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Synthesis of Hydroxamic Acids and Its Characterization through Spectroscopic Techniques

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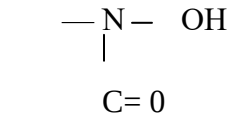
Abstract

Hydroxamic acids and their derivatives have attracted considerable attention, due to their pharmacological, toxicological and pathological properties. Hydroxamic acids generally have low toxicities and have a wide spectrum of activities in all types of biological systems. The present study deals with synthesis and characterization of a series of hydroxamic acids derived from benzoylation or acylation of substituted aryl hydroxylamine or hydroxylamine hydrochloride and elemental analysis, 1H NMR, 13C NMR, and IR spectral data of the compounds.

Keywords: Hydroxamic acid, Benzoylation, Acylation.

Introduction

Hydroxamic acids are one of the most extensively studied chemical compounds due to their tremendous applications in various fields. These are a class of chemicals in which a hydroxylamine is inserted into a carboxylic acid. Structure of hydroxamic acid is represented as R–CO–NH–OH, where R is an organic residue, CO is a carbonyl group, and hydroxylamine as NH2–OH (Agarwal and Kunji 2005).



Types of Hydroxamic Acids

Various types of hydroxamic acids have been reported so far by various researchers in the past. Different hydroxamic acids have different properties which are due to the type of acyl group and the length of carbon chain. Depending upon the length of carbon chain, hydroxamic acids can be classified as follows:

(a) Short-chain hydroxamic acids - Short-chain hydroxamic acids have a carbon chain of C2–C3. These are easily soluble in water, but some of the hydroxamic acids with very high chelating properties are used in resin synthesis, e.g., acetohydroxamic acid and propionohydroxamic acid (Sharma *et al.*, 2012).

(b) Middle-chain hydroxamic acids - Middle-chain hydroxamic acids have carbon chain of C4–C8. Generally these are aminohydroxamic acids. These are very useful in medical field since they are used to inhibit various metalloproteases. Potential applications of hydroxamic acids, such as butyrohydroxamic acid, valerohydroxamic acid, succinylhydroxamic acid, benzohydroxamic acid, nicotinylhydroxamic acid, homocysteinehydroxamic acid, suberoylanilidehydroxamic acid (SAHA), etc., have been well established in various processes (Celine and Kelvin 2000).

(c) Fatty hydroxamic acids or long-chain hydroxamic acids - These are generally long carbon chains of more than C12. These are insoluble in water and are very useful for removing toxic metal ions present in water. These are used as surfactants in detergent industry and also exhibit antibacterial and antifungal activities, e.g., actinonin, mycobactin, phenylalanylhydroxamic acid, lauryl hydroxamic acid, palmitylhydroxamic acid, 2,4-dihydroxy-1,4- benzoxazin-3-one (DIBOA), 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), etc. (Suhendra*et al.*, 2005; Haron*et al.*,2012).

Properties

Agrawal and tendon have determined the ionization conslants (pKa) of ten N-aryl hydroxamic acids in aqueous media,they found that at 25C° the pka values are in the range of 8.08-8.59 ,where at 35C° the pka values range from 8.01- 8.56 . Hydroxamic acids are stronger than phenol (pka=9.89), since they are differ widely in their structer and basicity. The acidity of hydroxamic

acids may be attributed mainly to the -OH group and it`s supression to intra molecular hydrogen bonding .Cyclic hydroxamic acids in the solid state , ,are capable of intramolecular(V III)

Synthesis of Hydroxamic Acid

Hydroxamic acid can be synthesized both by chemical and by enzymatic processes

Chemical Method: Chemically, hydroxamic acids can be synthesized by a number of reactions involving acids, esters, aldehydes, etc., detailed below.

From Acids: Different types of carboxylic acids have been successfully used to synthesize variety of hydroxamic acids. Since Loosen arrangement is one of the key methods for their synthesis, hence, ultrasonication is done for the accelerated production of these valuable acids (Vasantha*et al.,* 2010).

From Esters: Ethyl or methyl carboxylic esters can be converted into the corresponding hydroxamic acids. Hydroxylamine when mixed with esters in the presence of a base under suitable conditions produced hydroxamic acid in good quantity. This method has been frequently used for their production without loss of their stereochemical integrity (Massaro*et al.* 2007; Riva *et al.,* 2009).

Mechanism of Enzymatic Synthesis of Hydroxamic Acid

Amidase exhibit “Bi-bi Ping-Pong” mechanism for the acyl transfer activity. Amides first react with the enzyme to give acyl-enzyme complexes (E–S complexes)which subsequently leads to formation of carboxylic acids. If a strong nucleophilic agent like hydroxylamine is present instead of water (in case of acyltransfer activity) then its interaction with E–S complex results in the productionhydroxamic acids. After the formation of the product, the enzyme retains itsoriginal state and is ready to convert another molecule of amide and hydroxylamineto hydroxamic acid (Pandey *et al.,* 2011; Sharma *et al.,* 2012).

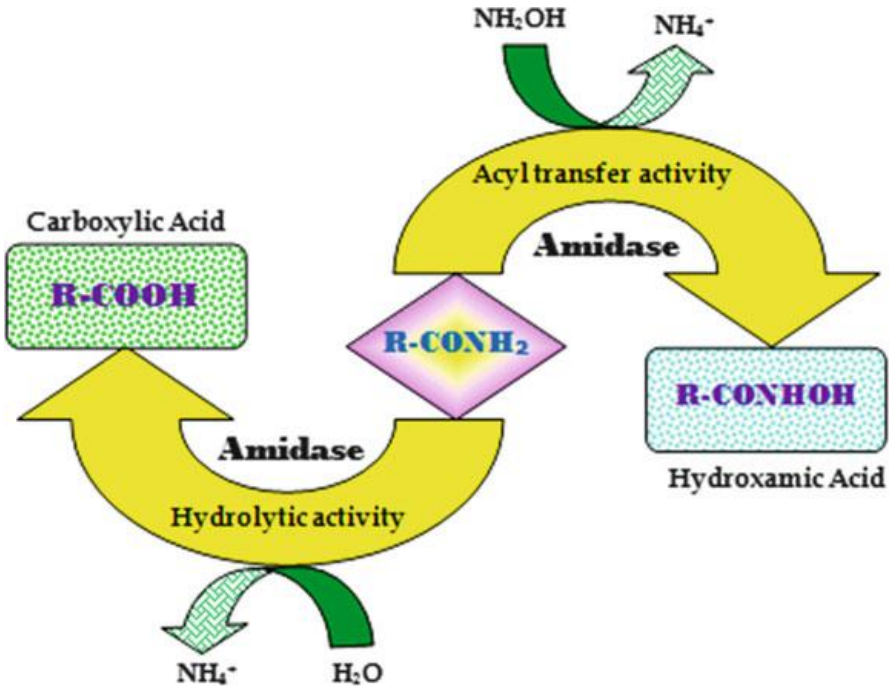


Fig. 1Types of reactions catalyzed by amidases (Bhatia *et al.*,2013)

Experimental

Reagents and chemicals

All chemicals used in the present investigation were of analytical grade and SHA was purchased from Sigma-Aldrich. All the solvents were dried and then distilled out. Doubly distilled water was used to prepare the required solutions.

Physical Measurements

Elemental analyses (C, H, N) of hydroxamic acids were performed.The melting points were determined in open capillary tubes using Prefit model. The molecularweight was determined by cryoscopic method using glacial acetic acid as solvent. The spectral studies of hydroxamic acids were carried out for its characterization using FTIR spectrophotometer (model 8400S, Schimadzu) and 300.4 MHz FT NMR spectrometer (model Jeol AL 300). The infrared spectra were recorded in KBr wafer phase and 1HNMR were recorded in CDCl3 using TMS as an internal standard.

Synthesis

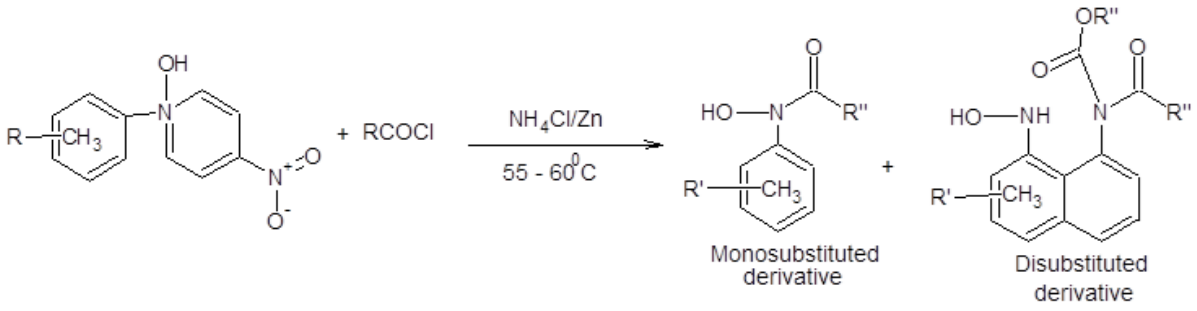
A procedure similar to that described by Priyadarshini and tandon was used. The details of the synthesisare described below which involves two stages:

Preparation of substituted N-phenyl hydroxylamine

Ammonium chloride (0.23 mol), substituted nitrobenzene (0.2 mol) and water (500 ml) were taken in a one litre beaker fitted with a thermometer and a mechanical stirrer. The mixture was stirred vigorously. Zinc dust (0.51 mol) was added in small amounts. The reaction being exothermic, the addition of zinc dust was so adjusted that the temperature did not exceed 55–60 ◦C. The stirring was further continued for about 20 min for complete reduction, till the temperature began to fall. The reaction mixture was filtered under suction and the filtrate was saturated with common salt in a conical flask. It was cooled in an ice bath for about 45 min to ensure maximum crystallization of substituted N-phenyl hydroxylamine.

Preparation of substituted hydroxamic acid

0.1 mol of the prepared hydroxylamine in 25 ml of benzene, distilled water and sodium bicarbonate 0.02 mol were taken in a 250 ml Erlenmayer's flask. Benzoyl chloride, 0.25 mol, was gradually added to the solution, with constant shaking, till effervescence ceased. The water layer was kept alkaline to litmus by the gradual addition of sodium bicarbonate. Addition of benzoyl chloride changed the colour of the reaction mixture from yellow to pink near completion of the reaction. Approximately 90 minutes are required for completion of the reaction. Both mono- and di-substituted derivatives are formed. The solution was filtered, and the solid was washed with water. The residue was purified using aqueous ammonia to remove di-substituted derivatives. The filtered ammonical solution, which was generally yellow or green, was added dropwise to slight excess of dilute sulphuric acid containing some crushed ice to yield the hydroxamic acid which was filtered, washed with water, and dried. The product wascrystallized from 60:40 (v/v) ethyl alcohol-water mixture. For BHA direct benzoylation of hydroxylamine hydrochloride and for AHA the acylation of hydroxylamine hydrochloride was carried out. The physical and analytical data obtained for these compounds are shown in table 1.



Where; R'= N, (p-CH_3), (o-CH_3), (m-Cl), (p-COOH), (p-OH),

R'' = C_6H_5, CH_3, C_6H_5 (o-OH)

Scheme 1

RESULTS AND DISCUSSION

The air- and light-stable compounds were synthesized by the reactions of benzoyl chloride with the appropriate hydroxylamine as shown by the scheme below.

Where, R´ = H, (*p*-CH3), (*o*-CH3), (*m*-Cl), (*p*-COOH), (*p*-OH);
R" = C6H5, CH3, C6H5 (*o*-OH)

Infrared spectra

The most characteristic bands associated with the hydroxamic acid functional group are due to the O–H and C=O stretching vibrations and these can be assigned rather unambiguously. Hydroxamic acids are characterized in the solid state by the bands between 3200-3150 cm-1 (O–H)29, a band near 1640 cm-1 (C=O)30, a band near 1599 cm-1 (C–N–C), a variable intensity band at 1440–1360 cm-1 (C–N), and a strong band29 near 900 cm-1 (N–O).

Table 1: Physical and Analytical data of substituted hydroxamic acid

S.N.	R´	R´´	Molecular Formula	Molecular Weight Found	Molecular Weight Calculated	Yield %	m.p.
1.	C6H5	C6H5	C13H11NO2	226	213	60	116

2.	C6H5 (*p*-CH3)	C6H5	C14H13NO2	241	227	65	102
3.	C6H5 (*o*-CH3)	C6H5	C14H13NO2	244	227	55	104
4.	C6H5 (*m*-Cl)	C6H5	C13H10ClNO2	263	247.5	57	106
5.	C6H5 (*p*-COOH)	C6H5	C14H11NO4	269	257	44	230
6.	H	CH3	C2H5NO2	97	75.00	62	82
7.	H	C6H5	C7H7NO2	152	137.14	57	123

Table 2: Analytical data of substituted hydroxamic acid

% Found			**% calculated**			**R_f**
C	**H**	**N**	**C**	**H**	**N**	
73.96	4.78	7.06	73.24	5.16	6.57	0.466
74.53	6.32	5.65	74.0	5.73	6.17	0.493
73.45	5.21	6.58	74.0	5.73	6.17	0.533
63.96	3.62	6.32	63.03	4.04	5.66	0.506
70.55	4.02	4.84	65.37	4.28	5.45	0.374
32.68	5.47	19.36	32.0	6.67	18.67	0.465
60.48	4.51	11.35	61.27	5.10	10.21	0.533

Assignment of the bands is given in table 3, in which the most intense bands are analyzed. There is large conjugation in the molecule under study, some deviations have been observed from the expected values. Hydroxamic acids are involved in strong hydrogen bonding which causes a large shift (of the order of 500 cm-1) in the absorption band to lower frequencies, and may be ascribed to resonance stabilization. A consequence of this resonance stabilization should be to increase the contribution of the single bond form, thereby causing a fall in the frequency of the C=O stretching vibration.

Nuclear Magnetic Resonance Spectra

The 1H NMR spectra of hydroxamic acids under investigation show the characteristic singlet of the proton of the hydroxyl group attached to the nitrogen atom in the region 10.5-11.5 ppm. The shifting of the resonance signal of hydroxyl proton to lower field supports intermolecular hydrogen bonding. The proton of –COOH in PCBHA is off scale which gives singlet between 10-11 ppm. A broad signal in the region 10.4-11.4 ppm which evidently belonged to the NH and OH protons of the hydroxylamine unit31. This suggests that OH and NH groups can undergo rapid proton exchange with each other.

Table 3: Infrared spectral data of substituted hydroxamic acid

S.N.	**R´**	**R´´**	**νO-H (cm-1)**	**νC=O (cm-1)**	**νN-O (cm-1)**	**νN-C (cm-1)**	**δC=C (cm-1)**	
1.	C6H5	C6H5	**3110(br)**	**1630**	**920**	**1400**	**765**	**690**
2.	C6H5 (*p*-CH3)	C6H5	**3110(br)**	**1640**	**920**	**1400**	**830**	**--**
3.	C6H5 (*o*-CH3)	C6H5	**3110(br)**	**1640**	**920**	**1390**	**770**	**--**
4.	C6H5(*m*-Cl)	C6H5	3250 (s)	**1620**	**940**	**1450**	**780**	**720**
5.	C6H5 (*p*-COOH)	C6H5	3300-2750 (br)	**1690**	**940**	**1420**	**840**	**--**
6.	H	CH3	3400-2750 (br)	**1660**	**990**	**1450**	**---**	**--**
7.	H	C6H5	3180 (br)	**1650**	**900**	**1450**	**795**	**680**

The 13C-NMR spectra exhibit absorption signal due to carbonyl, C=O carbon nearby 165 ppm. The chemical shifts of aromatic carbon appear in the region 138-121 ppm. Beside these signals, a singlet nearby 21 ppm and a singlet at 179 ppm appeared which correspond to the carbon atom of alkyl group and carboxy group respectively. Above NMR spectral data are summarized in table 4 and 5

Table 4: 1H NMR spectral data of substituted hydroxamic acid

S.No	R´	R´´	**δppm**	**Hydrogen**	**Multiplicity**	**Assignment**
1.	C6H5	C6H5	10.73 7.44-7.19	**1** **10**	Singlet Multiplet	O-H proton Aromatic protons
2.	C6H5 (*p*-CH3)	C6H5	10.67 7.43-7.02 2.32	**1** **9** **3**	Singlet Multiplet Singlet	O-H proton Aromatic protons CH3 protons
3.	C6H5 (*o*-CH3)	C6H5	10.32 7.55-7.13 2.38	**1** **9** **3**	Singlet Multiplet Singlet	O-H proton Aromatic protons CH3 protons
4.	C6H5 (*m*-Cl)	C6H5	10.89 7.45-7.01	**1** **9**	Singlet Multiplet	O-H proton Aromatic protons
5.	C6H5 (*p*-COOH)	C6H5	11.23 10.73 8.09-7.42	**1** **1** **9**	Singlet Singlet Multiplet	O-H proton Carboxy proton Aromatic protons
6.	H	CH3	10.42 (br) 2.58	**1** **3**	Singlet Singlet	O-H & N-H proton Methyl proton
7.	H	C6H5	11.19 (br) 7.90-7.26	**1** **5**	Singlet Multiplet	O-H & N-H proton Aromatic protons

Table 5: 13C NMR spectral data of substituted hydroxamic acid

S.No	R´	R´´	**13C NMR data**
1.	C6H5	C6H5	δ 165.26 (C-7), δ 139.42 (C-1), δ 132.05 (C-8), δ 131.03 (C-2, C-6), δ 129.10 (C-9, C-13), δ 128.87 (C-3, C-5), δ 128.19 (C-10, C-12), δ 125.98 (C-4, C-11)
2.	C6H5 (*p*-CH3)	C6H5	δ 165.20 (C-7), δ 138.48 (C-1), δ 136.92 (C-8), δ 132.15 (C-2, C-6), δ 130.89 (C-9, C-13), δ 129.75 (C-3, C-5), δ 128.89 (C-10, C-12), δ 128.13 (C-4), δ 126.15 (C-11), δ

			21.14 (C-14)
3.	C6H5 (*o*-CH3)	C6H5	δ 165.18 (C-7), δ 138.01 (C-1), δ 134.69 (C-8), δ 131.33 (C-2, C-6), δ 130.01 (C-13), δ 128.15 (C-3, C-5), δ 128.54 (C-12), δ 127.89 (C-10), δ 127.57 (C-4, C-11), δ 126.87 (C-9), δ 21.10 (C-14)
4.	C6H5 (*m*-Cl)	C6H5	δ 166.01 (C-7), δ 140.84 (C-10), δ 139.05 (C-8), δ 134.62 (C-1), δ 131.34 (C-9), δ 129.81 (C-11), δ 128.76 (C-12), δ 128.36 (C-6, C-2), δ 127.86 (C-13), δ 125.26 (C-5, C-3), δ 123.43 (C-4)
5.	C6H5 (*p*-COOH)	C6H5	δ 179.14 (C-14), δ 167.18 (C-7), δ 145.60 (C-11), δ 131.89 (C-10, C-12), δ 130.48 (C-8), δ 129.81 (C-9, C-13), δ 128.53 (C-1), δ 127.67 (C-2, C-6, C-3, C-5), δ 120.05 (C-4)
6.	H	CH3	δ 166.03 (C-1), δ 17.85 (C-2)
7.	H	C6H5	δ 165.37 (C-1), δ 132.11 (C-2), δ 131.25 (C-3, C-7), δ 128.57 (C-4, C-6), δ 127.71 (C-5)

Conclusion

The search for specific analytical reagents has led to the use of a large number of organic ligands for complex formation with metal ions. For the proper evaluation of analytical methods involving organic reagents it is necessary to know the nature, specificity, selectivity and sensitivity of the reactions with inorganic ions and the solubility and stability of the products of their reactions. Therefore, it is valuable to pursue the evaluation of versatile and important analytical methods. Organic reagents by their ease of substitution often afford reagents o f desired analytical properties. Hydroxamic acids, having the bidentate functional grouping (I), fulfil the basic requirement of complex formation with metal ions and, therefore, form an important family of chelating agents. Seven hydroxamic acids have been prepared and characterized on the basis of analytical and spectral data.

References

1. Agarwal YK, Kunji PS (2005) Synthesis and dissociation constant of calix (6) arenehydroxamic acid. Iran J SciTechnol 29:1-16.
2. Agrawal Y.K.andTandon S.G. (1973) "Electoanalytical chemistry and interfacial electrochemistry"43,158.
3. Bhatia RK, Bhatia SK, Mehta PK, Bhalla TC (2013) Production and characterization of acyl transfer activity of amidase from Alcaligenes sp. MTCC 10674 for synthesis of hydroxamic acids. J MicrobBiochemTechnol 5:001–005.
4. Celine JM, Kelvin BN (2000) Hydroxamic acids-ion chelators, aspirin analogues, nitric oxide donors and structurally diversed metals complexes.
5. Haron MJ, Jahangirian H, Yusof NA, Kassim A, Rafiee-Moghaddam R, Peyda M, Abdollahi Y, Amin J (2012) Benzyl and methyl fatty hydroxamic acids based on palm kernel oil a s chelating agent for liquid-liquid iron (III) extraction. Int J MolSci 13:2148–2159.
6. Keth, J.; Johann, T.; Frey, H. Hydroxamic acid: an underrated moiety? Marrying bioinorganic chemistry and polymer science. Biomacromolecules 2020, 21 (7), 2546−2556.
7. Massaro A, Mordini A, Reginato G, Russo F, TaddeiM(2007) Microwave-assisted transformation of esters into hydroxamic acids. Synthesis 12:3201–3204.
8. Pandey D, Singh R, Chand D (2011) An improved bioprocess for the synthesis of acetohydroxamic acid using DTT (dithiothreitol) treated resting cells Bacillus sp. APB-6. BioresourTechnol 102:6579–6586.
9. Priyadarshini and S.G. Tandon (1967). Journal of Chemical and Engineering Data: Vol. 12, 143.
10. Riva E, Gagliardi S, Mazzoni C, Passarella D, Rencurosi A, Vigo D, Martinelli M(2009) Efficient continuous flow synthesis of hydroxamic acids and suberoylanilidehydroxamic acid preparation. J Org Chem 74:3540–3543.
11. Sharma M, Sharma NN, Bhalla TC (2012) Biotransformation of acetamide to acetohydroxamic acid at bench scale using acyl transferase activity of amidase of Geobacilluspallidus BTP-5x MTCC 9225. Indian J Microbiol 52:76–82.

12. Suhendra D, Yunus WMZ, Haron MJ, Basri M, Silong S (2005) Enzymatic synthesis of fatty hydroxamic acid acids from palm oil. J Oleo Sci 54:33–38.
13. Vasantha B, Hemantha HP, Sureshbabu VV (2010) 1-Propanephosphonic acid cyclic anhydride (T3P) as an efficient promoter for the Lossen rearrangement: Application to the synthesis of urea and carbamate derivatives. Synthesis 17:2990–2996.