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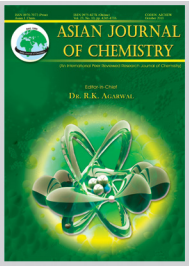

Studies on Synthesis and Kinetic Hydrolysis of Chiral Organic Phosphoramidates by Suitable Methods

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The study was focused on the nature of the substituents and their influence on the reactivity and the stability of the synthesized mixed chiral phosphoramidates. The *ortho*-chloro substituent has proved to be significant during hydrolysis resulting in maximum reactivity and also the presence of bulky groupings around the phosphorus atom hindered the approach of the nucleophile to a large extent, thereby resulting in the minimum rate of hydrolytic cleavage. The presence of dimethyl formamide has resulted in the deviation of the physiological conditions desired but no correlation could be tabled out for its influence on the rate of hydrolysis.

Key Words: Hydrolysis, Physiological conditions, Substituents, Phosphoramidates.

INTRODUCTION

In recent years, investigations on the synthesis of mixed organic phosphoramidates have become an important area in phosphorus chemistry. These compounds with peculiar structures have attracted attention because of their function as prodrugs[1-3], anti HIV[4-8] and antibacterial[9] agents. Phosphate esters play a dominant role in the physiology of cells and hence are essential to any organism. Most prominent is the participation of phosphate esters as a structural and functional element in DNA, RNA and its monomeric building blocks, the occurrence as a post-translational signal in proteins and as a head group in phospholipids. Most of the non-polymeric phosphate esters that occur in cells might be considered lead compounds for the development of drugs, as the majority of them are directly or indirectly involved in intracellular signaling or the assembly of DNA or RNA. Phosphate esters usually have a low pKa, often between 1 and 2. Subjected to the physiological pH in the range of 7.0-7.4, the compounds are permanently deprotonated and therefore negatively charged. The negative charge on phosphate esters is responsible for a variety of its properties[10].

Organophosphorus compounds (OP) still pose a major problem in toxicology. The use of organophosphorus pesticides for pest control and for attending suicides causes huge numbers of intoxications and several hundred of thousands of fatalities per year especially in developing countries. The main mechanism of action of organophosphorus compound is a progressive inhibition[11,12] of acetylcholinesterase (AChE) by phosphorylation of the active site serine leading to an inactive enzyme species. The determination of inhibition kinetics of organophosphorus compounds with human acetylcholinesterase showed a superior inhibitory potency of organophosphonates compared to organophosphates and phosphoramidates. These marked differences are in agreement with previous studies using electric eel, fetal bovine serum, bovine erythrocyte and brain acetylcholinesterase. The asymmetric synthesis of organophosphorus compounds is a relatively new field, which has developed mostly during the past three decades. The stereospecific enzymatic activity of the chiral organophosphate compounds is an important factor in the neurotoxicity of these compounds, the mechanism of which involves the inhibition of acetylcholinesterase to cause cholinergic effects or the inhibition and ageing of neuropathy target esterase[13] (NTE) to induce delayed neuropathy. Quite a number of review articles have summarized the work on phosphate prodrugs in the past. The bulk of work on prodrugs of phosphates was performed to allow delivery of biologically active nucleoside monophosphates to the cytosol as antiviral or anticancer drugs.

In the present paper, we performed the synthesis[14] of a series of mixed chiral[15] phosphoramidates and then their correlation study by hydrolytic[16,17] degradation under physiological conditions, keeping in mind about their pharmaceutical applications and drug discovery.

EXPERIMENTAL

Melting points were determined on an electric melting point apparatus and are uncorrected. The synthetic approach comprised of the conventional synthetic techniques of phospho-

rylation *via* the formation of methoxy phosphorodichloridate. Moreover, the progress of the reaction was monitored by TLC and further by gas chromatographic techniques. The hydrolytic study was performed by using Allen's modified method[18] in all the synthesized compounds under possible physiological conditions. All the materials used are of analytical grades and they are further purified and dried using reported methods.

General procedure: The mixed chiral phosphoramidates were synthesized according to the method described by Ibomcha *et al.*[14]. Thus, methanol was first reacted with phosphoryl chloride in dry benzene in the presence of triethylamine and formed the methoxy phosphorodichloridate (preparation zero). The methoxy phosphorodichloridate has been considered to be a safer phosphorylating reagent than $POCl_3$ itself and it also shows greater reactivity than the latter. Substituted phenol or aryl amine were further reacted with the more reactive phosphorodichloridate and obtained the desired chiral phosphoramidates by refluxing for 10-20 h depending on condition and product formed. Each compounds thus formed were isolated and purified using suitable solvent extraction methods. Compounds **1-9** with Cl-, F- or NO_2- substituents in different (*o*-, *m*-, *p*-) positions in aryl moieties, have been prepared *via* the stepwise procedure. Each member was checked for its melting point, solubility and stability parameters. It may be mentioned here that the following nine members of the mixed triesters (except the compound **9** where MeO- group is being replaced by *t*-$C_5H_{11}O$-) belonging to the category of organic phosphoramidates (P-chiral) have been synthesized: methoxy, phenoxy, N-phenyl phosphoramidate (**1**); methoxy, *p*-Cl-phenoxy, N-phenylphosphoramidate (**2**); methoxy, phenoxy, N-**p**-Cl-phenylphoramidate (**3**); methoxy, *o*-Cl-phenoxy, N-phenylphosphoramidate (**4**); methoxy, *p*-NO_2-phenoxy, N-*p*-F-phenyl- phosphoramidate (**5**); methoxy, *p*-Cl-phenoxy, N-*o*-Cl, *p*-NO_2-phenylphosphoramidate (**6**); methoxy, phenoxy, N-2-pyridylphosphoramidate (**7**); methoxy, *o*-*p*-Di-Cl-phenoxy, N-thiazolylphosphoramidate (**8**) and *t*-pentyloxy, 2-napthyloxy, N-*o*-Cl-, *p*-NO_2-phenyl phosphoramidate (**9**). Each member of the series was characterized by suitable spectroscopic techniques.

The quantitative chemical method was also applied for determining the percentage of phosphorus element in each compound and for this hydrolytic degradation studies were made with each member. All the nine numbers were subjected to the study of their reactivity during hydrolysis at 40 ± 0.5 ºC at pH 6.43, *i.e.*, near physiological conditions. The substrate concentration was kept as 8.0×10^{-4} M in each case and the medium, DMF, had to be varied from 0.0-8.0 % depending upon the solubility of each member. Allen's modified method[17] had been used for their quantitative estimation during the progress of hydrolysis in each case. It needs to be described that due to the presence of dimethyl formamide in varying percentages, the pH was as high as 9.83 in compound **4** when 8 % DMF had to be added in the reaction mixture during hydrolysis. In compound **3**, pH was again changed from 6.43-9.08 when 4 % DMF was employed for solubility reasons. On the other hand, in compound **8**, with 8 % DMF, the pH was only increased to 7.35. These changes in the pH values, as mentioned, do not directly correlate with DMF percentages. The genuine study was possible with compounds **5** and **7** only. In both these cases, the mixed (two C-O-P and one C-N-P linkage) triesters, the presence of N-*p*-F-anilino and N-*o*-NH_2-pyridyl units were a part of the compounds **5** and **7**, respectively, showing their special behaviour as far as solubility was concerned.

Detection methods: Infrared spectra were determined on a FT-IR spectrophotometer (Shimadzu PRESTIGE 21). ^{31}P NMR was recorded on a 400 MHz spectrophotometer and GC-MS was performed on Agilent Technologies Model 6890N and 5973.

Infrared and FT-IR absorption spectral studies on all the nine members showed the presence of the characteristic P=O (1320-1200 cm^{-1}); C-N-P (1100-800 cm^{-1}); P-O-C (arom.) [1260-1160 cm^{-1}] and P-O-C (aliph.) [1050-970 cm^{-1}]; along with the very characteristic P-N-H (str.) [3400-3010 cm^{-1}] and P-N-H(δ)[1580-1540 cm^{-1}] linkages. The observance of the bands in the above regions (reported) and their sharpness confirmed the presence of the desired bonding in the designed phosphoramidates.

^{31}P NMR studies were made with methoxy, phenoxy, N-phenyl phosphoramidate (compound **1**) and methoxy, phenoxy, N-*p*-Cl-phenyl phosphoramidate (compound **3**) clearly showed the single signal at 18.52 and 18.343 ppm; thus clearly justifying the structure of the compounds as belonging to the phosphoramidate type. It may also be mentioned that the reported range for organic phosphoramidates in ^{31}P NMR spectral studies is -25 to +50 ppm. The observed values fall in the reported range so that the evidence for their characteristic C-N-P linkage is obtained.

RESULTS AND DISCUSSION

TLC of the members indicated their purity in almost each and every case. GC-MS studies could be performed on compound **0**, compound **2** and compound **9**. These studies led to the presence of 62 % of the methoxy phosphorodichloridate (compound **0**) in the reaction mixture while the latter were determined during the progress of the synthetic reaction and could be estimated as 11 % (compound **2**) and 45 % (compound **9**) only.

First-order rate coefficients were calculated using the integral form of the rate law. The observed rate coefficients may or may not be influenced by the percentage of DMF. From the rate data, it has been seen that compound **4** (with an *o*-Cl-phenoxy component) exhibits the optimum rate of hydrolysis amongst the series of compounds undertaken for the hydrolytic study. This highest rate of hydrolysis in this case (compound **4**) may be attributed to the maximum percentage (8.0) of DMF. On the other hand, compound **9** constituted by *t*-pentyloxy, 2-naphthyloxy and N-*o*-Cl-*p*-NO_2-anilino components, hydrolyses with the least magnitude of the rate coefficient. In the latter member, the presence of bulky groupings around the phosphorus atom hindered the approach of the nucleophile to a large extent, thereby resulting in the minimum rate of hydrolytic cleavage.

Absence of DMF for compounds **5** and **7** decreases the rate of hydrolysis by nearly three times the optimum rate giving k_e obsd. = 1.61×10^{-3} min^{-1} and 1.30×10^{-3} min^{-1}, respectively.

These rate values clearly indicate that when DMF is present in the run, it may also act as a strong nucleophile, so that rates are accelerated as in compounds **3** and **4** mainly. However, no proper correlation could be established between the DMF percentage and the observed rates of hydrolysis (Table-1). Also, the presence of DMF resulted in deviation of the physiological conditions desired to be maintained for the designed P-epimeric (or mixed) triesters under observation. The following order of reactivity has, therefore, been arrived at, during the present study for the members examined (Fig. 1).

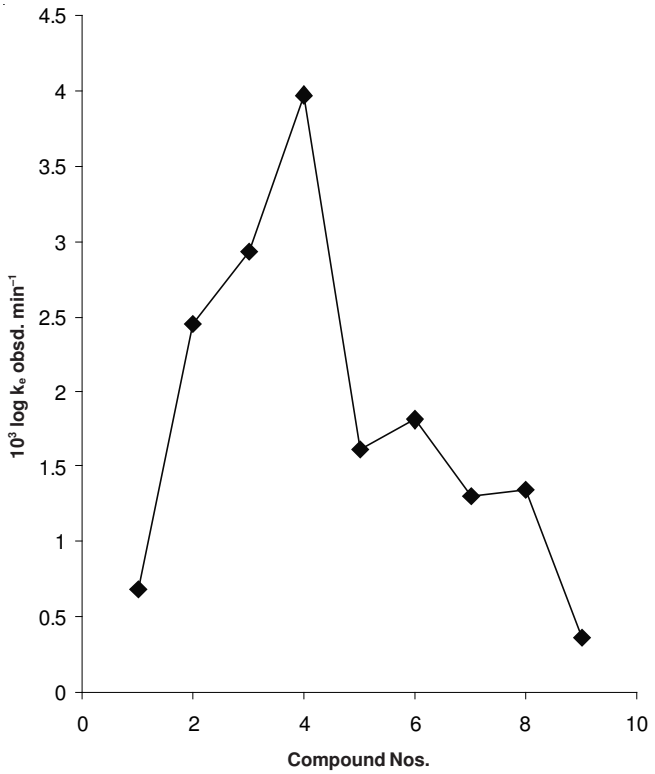


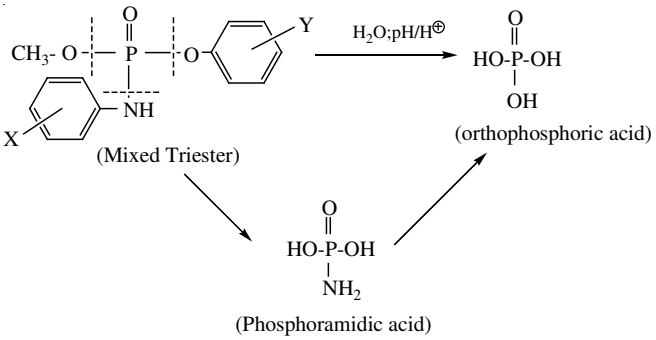
Fig. 1. Hydrolytic reactivity of nine synthetic organic phosphoramidates against their serial Nos. at pH 6.43 (*ca.*) in aqueous/DMF media at 40 ± 0.5 ºC

4 > 3 > 2 > 6 > 5 > 8 > 7 > 1 > 9

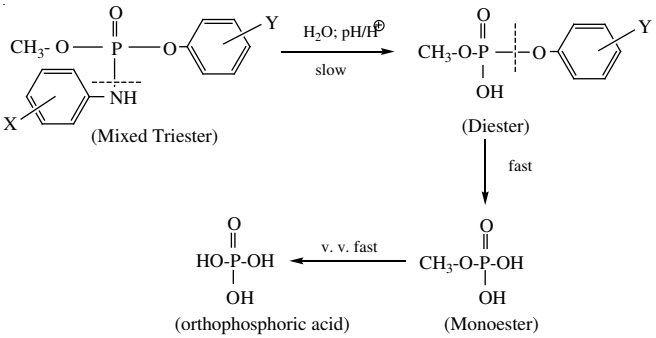
All the compounds consisted of substituents in the phenyl moieties connected *via* C-O-P and C-N(H)-P linkages. Out of the variety (Cl-, NO_2-, F-, NH_2- *etc*.) of groups present in *ortho*- and *para*- positions in simple aryl- or heterocyclic-components, an *ortho*-chloro substituent has proved to be significant during hydrolysis resulting in maximum reactivity of compound **4** (methoxy, *o*-Cl-phenoxy, N-phenyl phosphoramidate), in the presence of the highest percentage (8.0) of DMF.

Hammett[19] and Jaffe's[20] plots were also used to correlate the various functional groups for their reactivity in the series of organic phosphoramidates under observation. For this, cumulative values of various substituents present in aryl/heterocyclic moieties were used. Rho (ρ) was only 0.092 so that no suitable relationship was observed between the rate and the substituent included in the structures of the various members.

Mechanisms of bond cleavage during hydrolysis: The above hydrolytic study indicates two possible route of cleavage of the mixed triester. This can be represented graphically as given below (**Scheme-I** and **II**):



Scheme-I: Direct and simultaneous cleavage of all three linkages (two C-O-P and one C-N-P) *i.e*., a one step process



Scheme-II: Cleavage of the least stable C-N-P linkage *i.e*., a stepwise process

As can been seen that there can be a simultaneous cleavage of all the three linkages at a single step thereby leading to the final orthophosphoric acid residue. Similarly, in the stepwise route least stable C-N-P linkage is broken first followed by C-O-P containing the aryl matrix and finally the cleavage of

TABLE-1
KINETIC RATE DATA OF THE HYDROLYTIC REACTIVITY OF SYNTHETIC ORGANIC PHOSPHORAMIDATES (CPDS. **1-9**) AT pH 6.43 IN DMF AT 40 (± 0.5) ºC

Compound No.	Functional groups	DMF (%)	Actual pH	10^3 k_e min^{-1} obsd.	4 + log k_e
1	O-Me, O-H(Ph), N-H(Ph)	2.0	8.40	0.68	0.83
2	O-Me, O-*p*-Cl-Ph, N-H(Ph)	4.0	8.04	2.45	1.39
3	O-Me, O-H(Ph), N-*p*-Cl-Ph	4.0	9.08	2.93	1.47
4	O-Me, O-*o*-Cl-Ph, N-H(Ph)	8.0	9.83	3.97	1.60
5	O-Me, O-*p*-NO_2-Ph, N-*p*-F-Ph	0.0	6.99	1.61	1.21
6	O-Me, O-*o*-Cl-Ph, N-*o*-Cl, *p*-NO_2-Ph	4.0	9.05	1.81	1.26
7	O-Me, O-H(Ph), N-*o*-NH_2-Pyr.	0.0	6.50	1.30	1.11
8	O-Me, O-*o*, *p*-Di-Cl-Ph, NH_2-Thia.	8.0	7.35	1.35	1.13
9	O-*t*-Pent, O-Naph, N-*o*-Cl, *p*-NO_2-Ph	4.0	6.62	0.36	0.56

the most stable methoxy substituent. This is clearly projected from the kinetic study of the above nine members.

Conclusion

Organic phosphoramidates of the P-chiral type possessing both C-O-P (two) and C-N-P (one) linkages could be designed with the readily available raw materials. They did not require any phosphorylating reagent other than $POCl_3$ and the methoxy phosphorodichloridate proved as a versatile reagent for interaction with both the moderately acidic phenols as well as strongly basic aryl amines. The importance of an *o*-Cl- group has indicated its major role in enhancing their reactivity during hydrolysis. The resolution of these chiral compounds and their less toxic character are expected to pave way for their utility as pro-drugs mainly.

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