Oriental Journal of Chemistry Vol. **26**(1), 265-267 (2010)

Synthesis, identification and *in vitro* hydrolysis of a P-chiral heterocyclic phosphoramidate

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(Received: July 30, 2009; Accepted: August 28, 2009)

ABSTRACT

Methoxy, o, p-di-chlorophenoxy, N-2-aminothiazolyl phosphoramidate has been synthesized and checked for its stability as well as reactivity near physiological conditions but in vitro. The C-N-P ester hydrolyses at pH 6.43 in 8% DMF-H_2O mixture at 40(±0.5)^{0}C and its half-life was observed to be nearly 13 hrs. The magnitude of first-order rate co-efficients observed depends upon the presence of the thiazolyl and dichloro- phenyl moieties in it. The P-N bond fission was decided by product (azo-dye test) testing during the progress of hydrolysis. Synthesis was carried out in two steps via the formation of methoxy phosphorodichloridate. Structure was confirmed by GC-MS and IR studies.

Key words: Chiral phosphoramidate, hydrolysis, physiological conditions.

INTRODUCTION

Amongst the wide variety of organic compounds, chemistry of heterocyclics is quite interesting as well as has wide applicability. Natural products[1] like alkaloids, plant pigments, vitamins and drugs etc. along with nucleic acids, the biomolecules with heterocylic units in them, perform varied functions. The thiazole nucleus itself (a five-membered heterocycle with N and S in alternate positions, forms a part of the vitamin B_1 structure. Many valuable medicaments both natural and synthetic ones comprise of a heterocyclic moiety in them. Due to a large development in this field, heterocyclic chemistry has now become a separate branch of organic compounds. With this background, an organic phosphoramidate has been synthesized and it contains 2-aminothiazole in it. It was introduced that the introduction of a heterocyclic moiety in this compound might bring about some typical change in its behaviour particularly during hydrolysis.

EXPERIMENTAL

Methoxy, o, p-di-chlorophenoxy, N-2-aminothiazolyl phosphoramidate, a mixed alkyl, disubstituted-aryl, 2-aminothiazolyl derivative of phosphorus oxychloride has been synthesized in this chemical laboratory by a two-step phosphorylation procedure. The formation of methoxy phosphorodichloridate has been achieved from methanol with $POCl_3$ using CTAB as a catalyst in dry benzene[2] medium, by stirring at room temperature. In the latter step, 2-aminothiazole and 2, 4-dichlorophenol in 1:1 ratio (just like methanol) were added using quantitative presence of triethylamine and the reaction mixture was refluxed for nearly 16 hours. The washed and the recrystallised (acetone) product melted at 210-212^{0}C. Allen's modified method[3] was used to determine %age of phosphorus in the synthetic member.

%age of phosphorus = 9.14 (both theo. and obsd.)

RESULTS AND DISCUSSION

The synthesized P-chiral compound i.e. Methoxy, o, p-di-chlorophenoxy, N-2-aminothiazolyl phosphoramidate was characterized[4] and identified[4] by spectroscopic techniques. IR spectral data shows some characteristic bands: P-NH-R(aryl)-3333.99cm^{-1}; (P)-N-H -1593.66cm^{-1}; P=O -1279.58cm^{-1}; P-N-C -704.66cm^{-1}; P-O-C (arom.) -1174.93cm^{-1}; P-O-C(aliph.)-034.20cm^{-1} and this helps in the identification of the compound.

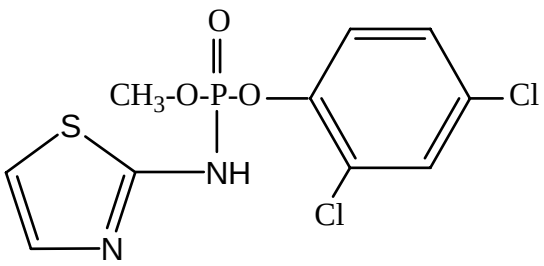
Using Bruker-Advance GC-MS technology[5], the mixed phosphoramidate was analyzed and found to contain a mixture of triester with two other minor components. However, TLC indicates a single spot. The major fragments (m/e) consisted of 239(3%)[339-100(probable loss of 2-NH_2-thiazole unit) =239]; 212(24%)[loss of CO]; 105(100%); 91 (48%) and 57 (20%).

On the basis of the above data from various physical (spectral) and chemical methods, Structure I is confirmed.

After preparing and purifying the mixed triester it was subjected to its hydrolytic degradation maintaining[6] a pH of 6.43(0.05M-KHP + 0.045M-NaOH). The study was made at 40(±0.5)^{0}C and the medium was kept as 8% DMF (for solubility reasons). These conditions of study may be considered as being quite close to physiological conditions[7]. During the hydrolysis, the quantitative method[3] given by Allen was used. The average pseudo-first order rate of hydrolysis is observed (Spectronic-20) to be 2.10 x 10^{-3} ke min^{-1}. A rate maximum was observed at t = 765 min. at 40(±0.5)^{0}C during the study. First-order rate co-efficients were calculated[8] using appropriate rate equation. Overall order is however, pseudo-first order due to the presence of excess medium. Actual pH was checked[5] during the progress of hydrolysis after heating for nearly 40 hours and it was found to be 7.35 i.e. higher than the actual pH maintained, and this was observed to be due to the presence of 8% DMF medium. The cleavage of P-N bond was also recognized by performing[2] the azo-dye test at appropriate time.

Comparison was also made in the hydrolytic rate data with the simplest mixed triester and the closely related one as shown in (Table 1).

Kinetic study thus reveals that the rate is quite fast as compared to the other two compounds as discussed above which may be due to the presence of two chlorine atoms in phenol along with the possibility of stabilization[10] (Structure I) due to the thiazole moiety.



Scheme 1: Methoxy, o, p-di-chlorophenoxy, N-2 aminothiazolyl phosphoramidate

Table 1: Comparison of hydrolytic rate data of related organic phosphoramidates at 40(±0.5)°C

Cpd.'s Name	Methoxy, phenoxy, N-phenyl phosphoramidate[9]	Methoxy, o,p-dichloro-phenoxy, N-2-amino-thiazolyl phosphoramidate[This work]	Methoxy, phenoxy, N-2-aminopyridyl phosphoramidate[9]
10^{-3} ke min^{-1}	0.68	< 2.10 >	1.3
DMF (%)	2.0	8.0	0.0
Actual pH	8.4	7.35	6.47

CONCLUSION

From the above experimental work, results and discussion, it is apparent that the combined phosphorylation of alcohol, phenol and arylamine results in a P-chiral system (GC-MS), which on dephosphorylation leads to simultaneous splitting of all the varied linkages under nearly physiological conditions.

ACKNOWLEDGEMENTS

One of the authors, Soram Ibomcha Singh is sincerely thankful to MPCST, Bhopal, for awarding fellowship as well as financial support.

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