Bull. Chem. Soc. Ethiop. **2014**, 28(1), 137-141.
Printed in Ethiopia
DOI: http://dx.doi.org/10.4314/bcse.v28i1.16
ISSN 1011-3924

SHORT COMMUNICATION

THE SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF A STABLE PHOSPHORUS YLIDE AND AN IMIDAZOLE AS NOVEL COMPOUNDS

Mina Ahmadi*, Rahebeh Amiri and Soutodeh Mohammadi

Islamic Azad University, Central Tehran Branch, Department of Chemistry, Faculty of Science, Tehran, Iran

(Received December 26, 2012; revised October 21, 2013)

ABSTRACT. A convenient one pot synthesis of two novel compounds including a stable phosphorus ylide and an imidazole from electron-poor acetylenes in fairly good yields by the condensation of triphenylphosphine and acetylene derivatives, in the presence of dimethyl thiourea from the 1:1:1 addition reactions is described. The structures of the synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis. The newly synthesized compounds were screened for antimicrobial on *Staphylococcus aureus*, *Bacillus sublitis, Escherichia coli* and *Pseudomonas aeruginosa* and antifungal activities on *Candida albicans,* patient isolate *Candida glabrata* and *Candida krusei*. The results showed that these compounds have activity against all the tested bacteria and fungi with a sufficient minimum inhibitory concentration.

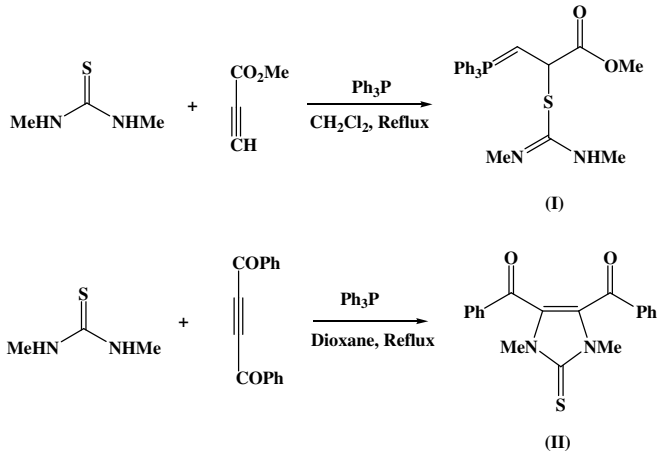
KEY WORDS: Phosphorus ylides, Electron-poor acetylenes, Imidazole, Antifungal, Antimicrobial

INTRODUCTION

The chemistry of phosphorus ylides is very significant in the twentieth century which takes part in many valuable reactions in organic synthesis because of its applications in the synthesis of organic products [1-5]. Phosphorus ylides are interesting synthetic targets because of their importance in a variety of industrial, biological and chemical synthetic usages. These compounds have pharmaceuticals properties, are widely used as anti-inflammatory, anticancer, analgesic, and antimicrobial activity [6-10]. Imidazole nuclei are also an important heterocyclic ring and a wide variety of imidazole derivatives are known for their chemotherapeutic importance especially antifungal activity [11-13]. Phosphorus ylides are reactive systems and several methods have been developed for the preparation of phosphorus ylides [4, 14, 15]. In general, phosphorus ylides are usually prepared by deprotonation of phosphonium salts which can be prepared most often by the reaction of triphenylphosphine and an alkyl halide [16-18] that often obtained in excellent yields from the 1:1:1 addition reaction between triphenylphosphine, dialkyl acetylendicarboxylates, in the presence of CH, SH, NH or OH-acid. Phosphonium salts can also prepared by Michael addition of phosphine to activated olefins. Michael addition of phosphorus(III) compounds such as triphenylphosphine to acetylenic esters leads to reactive 1,3-dipolar intermediate betaines which are not detected even at low temperature [4, 19, 20].

Here we describe a rapid and efficient one-pot synthesis method for the preparation of a stable phosphorus ylide (**I**) and an imidazole (**II**) using triphenylphosphine, acetylene derivatives (methyl acetylene carboxylate and dibenzoyl acetylene, respectively) and dimethyl thiourea (Scheme 1) and then synthesized compounds are tested for evaluation of their antibacterial and antifungal activities, respectively.

*Corresponding author. E-mail: ahmadi.mina63@gmail.com



Scheme 1. Synthesis of phosphorus ylides **I** and imidazole **II**.

EXPERIMENTAL

Material and equipment. All the chemicals and solvents were obtained from E-Merck (Darmstadt, Germany), and were used without further purification. All melting points are uncorrected and were taken with an electrothermal melting point apparatus (Electrothermal Eng. Ltd, Essex, UK). IR spectra were determinate in KBr on a Shimadzu Dr-8031 instrument. The ^{1}H-NMR and ^{13}C-NMR spectra of the synthesized compounds were measured in $CDCl_3$ or DMSO-d_6 solution and TMS as the internal standard using a Bruker 500 MHz and Advance-Bruker 300 MHz instruments, respectively. All chemical shifts were reported as δ (ppm) values. Elemental analyses were carried out using a Perkin-Elmer, CHN elemental analyzer model 2400 and were within ± 0.4% of the theoretical values.

2-(N,N-Dimethyl-carbamimidoylsulfanyl)-3-(triphenyl-λ^5-phosphanylidene)-propionic acid methyl ester (**I**). To a mixture of 0.312 g (3 mmol) of 1,3-dimethyl-thiourea and 0.252 g (3 mmol) methyl acetylene carboxylate in 5 mL dichloromethane, the 0.834 g (3 mmol) of triphenylphosphine was added. The resulted mixtures were stirred and heated under reflux for 2 h. After completing the reaction (monitored by TLC), the desired compound was obtained by solvent evaporating. The yellow precipitate re-crystallized from 1:1 ethyl acetate-n-hexane mixture. Yield 85%, m.p.: 123-124 ^{o}C; IR (KBr, ν_{max} cm^{-1}): 1068 (C-N), 1126, 1277 (C-O), 1382 (CH_3), 1458, 1600 (C=C aromatic), 1729 (C=O), 2929 (C-H), 3068 (=C-H), 3413 (-NH); ^{1}H NMR ($CDCl_3$, δ ppm): 2.68 (3H, s, =NMe), 3.80 (3H, d, HNMe), 3.82 (3H, s, OMe), 6.08-6.15 (1H, d, C-H, 1H, d, =C-H), 7.09-7.77 (15H, aromatic); ^{13}C-NMR ($CDCl_3$, δ ppm): 29.71 (P=CH), 30.96 (HNMe), 31.24 (CH), 38.15 (=NMe), 59.52 (OMe), 128.24-141.65 (aromatic), 167.73 (N=C), 169.71 (C=O). Anal. calcd. for $C_{25}H_{27}N_2O_2PS$: C, 66.66; H, 6.01; N, 6.22. Found: C, 66.39; H, 5.90; N, 6.97.

(1,3-Dimethyl-2-thioxo-2,3-dihydro-1H-imidazol-4,5-diyl) bis (phenyl-methanone) (**II**). To a mixture of 0.104 g (1 mmol) of 1,3-dimethyl-thiourea and 0.234 g (1 mmol) dibenzoyl acetylene (DBA) in 5 mL dioxane, the 0.278 g (1 mmol) of triphenylphosphine was added. The resulted mixtures were stirred and heated under reflux for 2 hours. After completing the reaction (monitored by TLC), the desired compound was obtained by solvent evaporating. The yellow precipitate re-crystallized from 4:1 ethyl acetate-n-hexane mixture. Yield 80%, m.p.: 145-148

^{o}C; IR (KBr, ν_{max} cm^{-1}): 540, 727 (=C-H aromatic (mono subst. oop)), 1109 (C-N), 1191 (C=S), 1409-1600 (C=C aromatic, alkene), 1699 (C=O), 2909 (C-H), 3087 (=C-H); ^{1}H NMR ($CDCl_3$, δ ppm): 2.16 (6H, s, NMe), 7.47- 7.68 (10 H, aromatic); ^{13}C-NMR ($CDCl_3$, δ ppm): 29.81 (NMe), 128.43- 132.17 (aromatic), 133.21 (C=C ethylene), 157.30 (C=S), 188.76 (C=O). Anal. calcd. For $C_{19}H_{16}N_2O_2S$: C, 67.86; H, 4.76; N, 8.33. Found: C, 67.20; H, 4.45; N, 8.10.

Screening for antibacterial activity. The antimicrobial activities were determined using disc diffusion method [21] by measuring the zone of inhibition in mm. Newly synthesized compound was screened *in vitro* for its antibacterial activity against two Gram-positive strains (*Staphylococcus aureus* and *Bacillus sublitis)* and two Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa)* at concentration of 500 µg/mL. Ciprofloxin (10 µg/disc) was used as a standard drug for antibacterial screening. New synthesized compound exhibited sufficient antibacterial activities. Each experiment was done in triplicate and the average reading was taken. The results are tabulated in Table 1.

Table 1. Results of antimicrobial activity of the tested compound.

Compound	Antibacterial activity			
	S.aureus	*B.subtilis*	*E.Coli*	*P.aeruginosa*
$C_{25}H_{27}N_2O_2PS$	++	++	-	-
Ciprofloxacin	+++	+++	+++	+++

Key to symbols: Highly active = +++ (inhibition zone > 12 mm). Moderately active = ++ (inhibition zone 9–12 mm). Slightly active = + (inhibition zone 6–9 mm). Inactive = - (inhibition zone < 6 mm).

Screening for antifungal activity. The yeasts *Candida albicans*, patient isolate *Candida glabrata* and *Candida krusei* were grown on Sabouraud Dextrose Broth (Difco); the yeasts were incubated for 48 h at 25.91 ^{o}C. The antifungal activity test was carried out at pH 7.4 in Sabouraud Dextrose Broth and the 2-fold dilution was applied. A set of tubes containing only inoculated broth was kept as controls. After incubation for 48 h at 25.91 ^{o}C, the last tube with no yeast growth was recorded to represent minimum inhibitory concentration (MIC), expressed in µg/mL. The results are given in Table 2. Antifungal activities of imidazole compound were carried out by Disc Diffusion Technique [22].

RESULTS AND DISCUSSION

Chemistry. On the basis of the well established chemistry of trivalent phosphorus nucleophiles it is reasonable to assume that phosphorus ylides result from the initial addition of triphenylphosphine to the electron-poor acetylenes and a concomitant protonation of the 1:1 adduct by dimethyl thiourea. The reaction between methyl acetylene carboxylate and 1,3-dimethyl-thiourea in the presence of triphenylphosphine proceeded in dichloromethane and dioxane and was complete after 2 hours. The yellow powders separated from the reaction mixture were identified as phosphorus ylide (**I**) and imidazole (**II**). The ^{1}H and ^{13}C NMR spectra of the crude product clearly indicated the formation of stable phosphorus ylide and imidazole. No product other than **I** and **II** could be detected by NMR spectroscopy. The structures of compounds **I** and **II** were deduced from their elemental analyses, IR, ^{1}H and ^{13}C NMR spectra. The ylide moiety of these compounds is strongly conjugated with the adjacent carbonyl group.

The ^{1}H NMR spectrum of **I** exhibited singlet (for CH_3-N=, imine group, δ = 2.68 ppm) and (for CH_3-O-, methoxy group, δ = 3.82 ppm), doublet (for CH_3-NH-, amine group, δ = 3.80 ppm) and (for C-H and =C-H, δ = 6.08-6.15 ppm). The phenyl residues gave rise to characteristic signals in the aromatic region (δ = 7.09-7.77 ppm) of the spectrum. The ^{1}H NMR spectrum of **II**

exhibited singlet (for CH_3-N, imide group, δ = 2.16 ppm). The phenyl residues gave rise to characteristic signals in the aromatic region (δ = 7.47-7.68 ppm) of the spectrum.

The ^{13}C NMR spectrum for **I** showed eight distinct resonances and for **II** showed five distinct resonances, as expected for the phosphorus ylide and imidazole structures. Partial assignment of these resonances is given in the Experimental section. The structural assignment of compound **I** and **II** made on the basis of the ^{1}H NMR and ^{13}C NMR spectra is supported by its IR spectrum, which in the carbonyl region displayed distinct absorption bands for the stretchings of the C=O groups.

Pharmacology. To check the biological activity of the compounds, the two compounds **I** and **II** were screened for *in vitro* antimicrobial activity and antifungal against a variety of bacteria and fungi. The antimicrobial activity was determined using disc diffusion method [21] by measuring the zone of inhibition in mm. The compound **I** was assayed for antibacterial activity against two Gram-positive strains (*Staphylococcus aureus* and *Bacillus sublitis)* and two Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa)*. From the data presented in Table 1, the preliminary screening results for the compound **I** established that compound **I** was showing a sufficient activity against all the tested bacteria.

The *in vitro* antifungal activity of the compound **II** was tested by the tube dilution technique [22]. The test compound and standards Miconazole, Fluconazole and Cotrimoxazole were dissolved in 10% DMSO, at concentrations of 100 µg/mL. Further dilutions of the compound and standards in the test medium were prepared at the required quantities of 50, 25, 12.5, 6.25, 3.125, 1.5 and 0.78 µg/mL concentrations. The final inoculums size was 10^5 CFU/mL. The MICs were defined as the lowest concentrations of the compounds that prevented visible growth. It was determined that the solvent had no antifungal activity against any of the test microorganisms. All the compounds were tested for their *in vitro* growth inhibitory activity against *C. albicans*, patient isolate *C. glabrata* and *C. krusei* (Table 2). Compounds **II** possessed comparable activity to Fluconazole and Cotrimoxazole against *C. albicans* with a MIC of 12.5 µg/mL. However the compound was not superior to the standards used against any fungi.

Table 2. Antifungal activities of the synthesized compound (MIC, µg/mL).

Compound	*C. albicans*	*C. glabrata*	*C. krusei*
$C_{19}H_{16}N_2O_2S$	12.5	12.5	12.5
Fluconazole	12.5	3.125	3.125
Miconazole	6.25	3.125	1.5
Cotrimoxazole	12.5	3.125	3.125

CONCLUSION

The present method may be considered as a practical route for the synthesis of the stable phosphorus ylides and imidazole using a one-pot reaction between triphenylphosphine and acetylenes in the presence of NH compounds such dimethyl thiourea. This procedure has advantages of high yield, mild reaction conditions, and simple experimental and work-up conditions and may be an acceptable method for the preparation of phosphorus ylide and imidazole. Our studies clearly demonstrate that novel synthesized compounds had significant pharmaceutical properties. As a consequence, we can conclude that newly synthesized can be used for the development of new drugs in future.

ACKNOWLEDGEMENTS

This work was a research project at Islamic Azad University, Central Tehran-Branch, Iran to achieve an M.Sc. degree and the authors gratefully acknowledge support of the university. Authors thank Neda Ghasemipour for her assistance with the pharmacological tests.

REFERENCES

1. Wittig, G. *Science* **1980**, 210, 500.
2. Crayson, M.; Griffith, E.J. *Topics in Phosphorus Chemistry*, Insterscience: New York; **1972**; p 7.
3. Hudson, H.R.; *Primary, Secondary and Tertiary Phosphines, Plyphosphines and Heterocyclic Organophosphorus Compounds*, in *Chemistry of Organophosphorus Compounds*, Vol. 1, Wiley: New York; **1990**, pp. 386-472.
4. Engel, R. *Synthesis of Carbon–Phosphorus Bond*, CRC Press: Boca Raton, Florida; **1988**.
5. Cadogan, J.I.G. *Organophosphorus Reagent in Organic Synthesis*, Vol. 12, Academic Press: New York; **1990**; p 564.
6. Fitjer, L.; Quabeck, U. *Synth. Commun.* **1985**, 15, 855.
7. Ramazani, A.; Souldozi, A. *Phosphorus Sulfur Silicon* **2005**, 180, 2801.
8. Yavari, I.; Adib, M. *Tetrahedron* **2001**, 57, 5873.
9. Yavari, I.; Asghari, S. *Tetrahedron* **1999**, 55, 11853.
10. Islami, M.R.; Hassani, Z.; Sheibani, H.; Abdolahzadeh B.; Etminan, N. *Tetrahedron* **2003**, 59, 4993.
11. Can-Eke, B.; Puskullu, M.O.; Buyukbingol, E.; Ican, M. *Chemico-Biological Interactions* **1998**, 113, 65.
12. Kus, C.; Ayhan-Kilcigil, G.; Can-Eke, B.; Iscan, M. *Arch. Pharm. Res.* **2004**, 27, 156.
13. Ayhan-Kilcigil, G.; Ku, C.; Coban, T.; Can-Eke, B.; Lcan, M. *J. Enzyme Inhib. Med. Chem.* **2004**, 19, 129.
14. Johnson, A.W. *Ylide Chemistry*, Academic Press: London; **1966**.
15. Cadogan, J.I.G.; Organophosphorus Reagents in Organic Synthesis, Academic Press: NewYork, **1979**.
16. Shen, Y. *Acc. Chem. Res.* **1998**, 31, 584.
17. Cadogan, J.I.G. *Organophosphorus Reagents in Organic Synthesis*, Academic Press: New York; **1979**.
18. Johnson, A.W. in *Ylides and Imines of Phosphorus*, Wiley: New York; **1993**; a) p 166; b) p 174; c) p 179; d) p 199.
19. Arduago, A. J.; Stewart, C. *Chem. Rev.* **1994**, 94, 1215.
20. Wasserman, H.H.; Baldino, C.M.; Coats, S.J.J. *Org. Chem.***1995**, 60, 8231.
21. Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C.; Truck, M. *Am. J .Clin. Pathol.* **1996**, 45, 496.
22. Sahm, D.F.; Washington, J.A. *Antibacterial Susceptibility Tests: Dilution Methods* in *Manual of Clinical Microbiology*, 5th ed., Balowes, A.; Hausler, W.J.; Hermann, K.L.; Shadomy, H.D. (Eds.), American Society for Microbiology: Washington DC; **1991**, p 1105.