Chemical and Process Engineering Research
ISSN 2224-7467 (Paper) ISSN 2225-0913 (Online)
Vol.10, 2013

Synthesis and Characterization of Polycyclicacetal Derived from PEG & Erythro-Ascorbic Acid Derivative and Study Its Effect on the Activity of ACh Enzyme (In Vitro)

Maha A. Younus
Department of Chemistry.Ibn -Al-Haithem College of Education for pure science
Baghdad University -IRAQ
E -mail: ma2004ha@yahoo.com

Abstract
Polycyclicacetal was prepared from the reaction of PEG with aldehyde derived from Erythro-ascorbic acid (pentulosono-ɣ-lactone-2,3-enedianisoate).All these compounds were characterized by Thin Layer Chromatography (TLC) and FTIR spectra and aldehyde was also characterized by (U.V-Vis), ^{1}HNMR, ^{13}CNMR, and mass spectra.The inhibitory effect of prepared polymer on the activity of human serum AcetylCholinesterase has been studied in vitro. The polymer showed a remarkable activity at low concentration ($4.7x10^{-3}$ – $4.7x10^{-8}$ M).
Key words: Polycyclicacetal, PEG, Acetylcholinesterase.

1. Introduction

In recent years, more interests have been emphasized in the synthesis of polymers containing polycyclicacetal segments, because of the ease of degradation of these polymers under mild conditions by treatment with a trace of acid (1).
Polyethylene glycol (PEG) is finding a rapidly expanding use in biochemical and bio medical applications. It has been found to be non-toxic, non-immunogenic and water soluble. PEG has therefore been used in protein modification to decrease antigenicity, prolong its plasma circulatory half-life and to increase its solubility and thermal stability (2-5)
L-ascorbic acid (LAA; i.e., vitamin C, a water soluble vitamin) contains a variety of biological, pharmaceutical and dermatological functions; for example, it can promote collagen biosynthesis, provide photoprotection, scavenge free radical, cause melanin reduction and enhances the immunity (e.g., anti-viral effect)(6-8). From the perspective in biochemistry, these functions are closely related to the so-called antioxidant properties of this compound.
Thousands of compounds have been synthesized and tested as a cholinesterase inhibitors. They belong to different types of organic and organometalic classes such as alkaloids, physostigmine, organophosphrous (9).
Acetyl cholinesterase (E.C.3.1.17) also known as AChE, is an enzyme which is critical to the function of animals from ants to elephants(10). This enzymes sole responsibility is to break down the neurotransmitter acetylcholine. Acetylcholine sends message between nerves, signaling muscle concentrations. If the neurotransmitter was not broken down after it had served its function, the muscle involved would not be able to relax, and this could create spasms, paralysis, and other problems(11,12). The enzyme acetylcholinesterase can be found in the synaptic cleft, the gap between nerve cells through which information flows. When acetylcholine passes through, the enzyme breaks it down into choline and acetic acid after it has served its function, ensuring that the neurotransmitter does not continue to float through the body. The choline and acetic acid are recycled by the body to make more acetylcholine so that reserves of neurotransmitter will be ready when the body needs it. Acetylcholinesterase can break down acetylcholine in microseconds, working rapidly to keep the synaptic cleft clear so that mixed message do not occur(13,14).

2. Experimental

2.1. Preparation of polycyclicacetal

Melting points were determined by electrothermal Stuart melting point apparatus and are uncorrected. IR spectra (in KBr) were recorded on 8400s Shimadzu FT infrared spectrophotometer. ^{1}HNMR spectrum was recorded on Ultra Shield (300 MHz) spectrometer with tetramethyl silane as internal standard. ^{13}CNMR spectrum was recorded on a Varian Mercury plus 100 MHz spectrometer. Electronic spectrum was obtained using a (U.V-Vis) spectrophotometer type CECl 7200 England. Mass spectrum was recorded on IEOLJMS-7high resolution instrument. Thin layer chromatography (TLC) were performed on aluminum plates coated with layer of silica gel, supplied by Merck. The spots were detected by iodine vapor. All chemical were obtained from Fluka or BDH.

Synthesis of 5,6-*O*-isopropylidene-L-ascorbic acid (2)

Dry hydrogen chloride was rapidly bubbled with stirring for 20 minutes into a (250ml) flask containing (10g, 57mmol) of powdered L-ascorbic acid (1) and (100ml) of dry acetone.

After addition of (80ml) n-hexane, stirring and cooling in an ice-water, the supernatant was decanted. The precipitate was washed four times with (154ml) of acetone-hexane mixture (4:7) (v/v), cooling in an ice-water and removal of supernatant after each addition. The last precipitate was dried under reduced pressure to give (2) (95.35%) as a white crystalline residue (15), m.p (206-208˚C). R_f (0.68) (benzene: methanol, 5:5) (v/v). FTIR (KBr, cm^{-1}): 3240 (O-H), 2993 (C-$H_{ali.}$), 2908 (C-$H_{ace.}$), 1751 (C=$O_{lac.}$), 1662 (C=C), 1431 (-CH_{asym}-), 1388 (-CH_{sym}-), 1141-900 (C-O), 767 δ(O-H) (O.O.P.) (16).

Synthesis of 2,3-*O*-dianisoyl-5,6-*O*-isopropylidene-L-ascorbic acid (3)

To a cold solution of (2) (10g , 46mmol) in pyridine (50ml), anisoyl chloride was added as dropwise (17.5ml , 129mmol) with stirring .The resulting mixture was stirred for 2 hours , then kept in dark place at room temperature for 22 hours.

The mixture was poured into ice-water and stirred for 20 minutes, the supernatant was decanted. The oil layer was extracted with chloroform (150 ml), washed with water, dilute hydrochloric acid (5%) (2 × 100ml.), saturated aqueous sodium hydrogen carbonate (100ml) and water. Dried over anhydrous magnesium sulfate, Chloroform was evaporated to produce a brown syrup and purified from chloroform: petroleum ether (60-80˚C) (1:5) (v/v) to give (3) (15g, 76.5%) as a pale yellow solid (17) , m.p (102-104˚C). R_f(0.80) (benzene: methanol, 5:5) (v/v). FTIR (KBr, cm^{-1}): 3028 (C-$H_{ar.}$), 2983 (C-$H_{ali.}$), 2939 (C-$H_{ace.}$), 2843 (OC-$H_{ali.}$), 1749 (C=$O_{lac.}$), 1683 (C=$O_{est.}$), 1647 (C=$C_{ali.}$), 1604 (C=$C_{ar.}$), 1300-1107 (C-$O_{est.}$), 900-600 δ(C-H) (O.O.P.).(16)

Synthesis of 2, 3-*O*-dianisoyl-L-ascorbic acid (4)

Compound (3) (10g , 23.6mmol) was dissolved in mixture (65%) acetic acid (30ml) and absolute methanol (10ml) and stirred for 48 hours at room temperature. The TLC showed that the reaction was complete (benzene: methanol, 6:4).

To the resulting solution a benzene (40ml) was added and evaporated (repeat this process four times)(17). The residue recrystallized from chloroform and then diethyl ether to yield (4) (7g, 77.7%) as a white crystals, m.p (130-132˚C), R_f (0.42). FTIR (KBr, cm^{-1}): 3444 (O-H), 3008 (C-$H_{ar.}$), 2972 (C-$H_{ali.}$), 2843 (OC-$H_{ali.}$), 1741 (C=$O_{lac.}$), 1681 (C=$O_{est.}$), 1647 (C=$C_{ali.}$), 1606 (C=$C_{ar.}$), 1319-1112 (C-$O_{est.}$), 900-600 δ(C-$H_{ar.}$) (O.O.P.)(16).

Synthesis of pentulosono-γ-lactone-2,3-enedianisoate (5)

To the stirred solution of sodium periodate (5.6g, 26mmol) in distilled water (60ml) at (0˚C), a solution of (4) (10g, 26mmol) in absolute ethanol (60ml) was added dropwise. After stirring for 15 minutes, ethylene glycol (0.5ml) was added as dropwise, stirring was continued at room temperature for 1 hour (17).

The mixture was filtered and to the filtrate water (40ml) was added then the product was extracted with ethyl acetate (3×50ml), the extracts dried by anhydrous magnesium sulfate, then filtered and the solvent was evaporated and the residue recrystallized from benzene to yield the pure product of compound (5) (4g, 45%) as a white crystals, m.p (156-158˚C). R_f (0.70) (benzene: methanol, 6:4) (v/v). FTIR (KBr, cm^{-1}): 3040 (C-$H_{ar.}$), 2983 (C-$H_{ali.}$), 2843 (OC-$H_{ali.}$), (2671, 2559) (C-$H_{ald.}$), 1782 (C=$O_{lac.}$), 1749 (C=$O_{ald.}$), 1685 (C=$O_{est.}$), 1604 (C=$C_{ar.}$), 1300-1107 (C-$O_{est.}$), 900-600 δ(C-$H_{ar.}$) (O.O.P.). ^{1}HNMR (DMSO δ ppm): 12.5 (s, 1H, CHO.), 7.00-7.97 (dd, 8H, aromatic), 3.86 (s, 1H, H_4), 3.82 (s, 6H, 2OCH_3)(16). ^{13}CNMR (DMSO δ ppm): 167.50 (C=$O_{lac.}$), 163.32 (C=$O_{est.}$), 131.86 (C-4), 131.83 (C-3), 131.81 (C-2), (123.44, 114.31, 114.28, 114.26) ($C_{ar.}$), 55.90 (OCH_3) (16). The signal of aldehydic carbonyl was disappeared due to it showed out of the scale(18). MS, (positive ion) m/z (relative intensity): 413 [M+1, (100)], UV (λ_{max}, nm, $CHCl_3$): 300.

Synthesis of polyethyleneacetal

The aldehyde (pentulosono-δ-lactone-2,3-enedianisoate) was dissolved in a mixture of benzene (8ml) and ethanol (2ml) with two drops of HCl. PEG (Mw = 4000, 0.5 g) was added to the mixture with vigorous stirring at (40 – 50)°C for 24 hr. The solution was poured into excess amount of methanol (100 ml) containing equimolar amount of NaOH, the product was separated by filtration and then washed with methanol and dried under vacuum. FTIR (KBr, cm^{-1}):3448 (O-H), 3057 (C-H_{ar}), 2954 (C-H_{ali}), 1597 (C=$O_{anisoate}$), 1279-1068 (-C-O-$C_{ace.}$), 923-680 δ(C-$H_{ar.}$)(16).

2.2. Enzyme assay

This compound was dissolved in dimethyl sulphoxide (DMSO) and stock solutions were made for this compounds. Different volumes from stocks were added to the assay mixture and the enzyme activity determined according to a slightly modified WHO procedure(19).

A volume of 2.250 ml of phosphate buffer pH= 7.2 , 50µl of DTNB solution and 10µl serum was served as the assay mixture. In a 3ml cuvette, 2ml of this mixture was taken and 34µl of the substrate (acetylthiocholineiodide) was added and the absorbance was measured at 430nm.

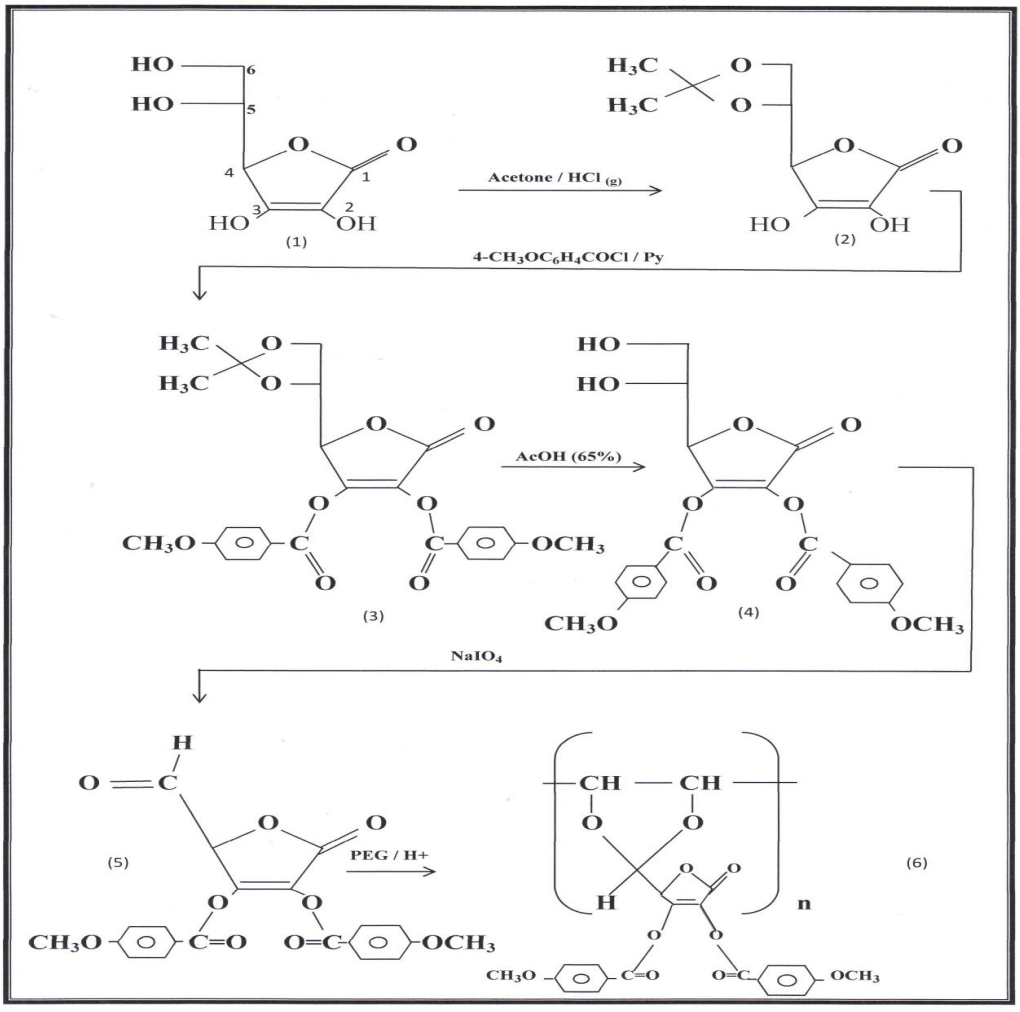
DMSO was used as a vehicle solution (control) and showed no inhibitory effect on the activity of the enzyme (20).

3. Results and Discussion

In the present work the synthesis of new polyacetal was achieved from pentulosono-γ-lactone-2,3-enedianisoate (5) , scheme (1). The first step employs the protection of the hydroxyl groups at C-5 and C-6 positions in L-ascorbic acid with acetal formation leading to compound (2) using dry acetone in acidic media, following Salomon (15) method. This is followed by esterification of the hydroxyl groups at C-2 and C-3 positions with excess of anisoyl chloride in dry pyridine.

The FTIR spectra for compound (2) and (3) were confirmed the formation of compound (3) by disappearance of the bands for (O-H) of compound (2) and exhibited the band at (1683) cm^{-1} for (C=O) of the ester in compound (3) spectrum.

In order to prepare aldehyde (5), the acetal moiety was cleaved under acidic condition (21) (65% acetic acid) for compound (3) to give (4) and oxidation of the product with sodium periodate to result (5), which gave a positive Tolen's test by formation a silver mirror (22). The FTIR spectra for compound (4) and (5) were confirmed the formation of compound (5) by disappearance of the bands for (O-H) of compound (4) and exhibited the band at (1749) cm^{-1} for (C=O) in compound (5) spectrum. The structure of (5) was confirmed by ^{1}HNMR which exhibited a signal at δ (12.5) ppm for (CHO) and was characterized by ^{13}CNMR and (U.V-Vis) spectrum which showed one peak at (300) nm (33333 cm^{-1}) assigned to ($\pi \longrightarrow \pi^*$) and ($n \longrightarrow \pi^*$) transitions. Finally, the mass spectrum showed a highest mass signal at [M+1] =413 with signal intensity 100%. The FTIR spectrum for compound (6) confirm the formation of the polyacetal by disappearance of the band (1749) cm^{-1} for ($C=O_{ald.}$) and the appearance of the band (1279-1068) cm^{-1} for ($-C-O-C_{ace.}$).



Scheme (1): The scheme of prepared polycyclicacetal.

3.1. Enzymatic study

The effect of polyethyleneacetal on serum cholinesterase activity invitro were carried . This compound shows an encouraging inhibitory action as compound to aknown inhibitors such as Dibucaine and NaF(23). The percentages of inhibition of this compound are shown in table (1):

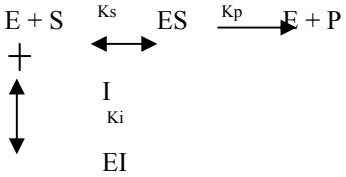
Table (1): The effect of different concentrations of polyethyleneacetal on (AchE) in serum.

Inhibitor Conc. (µ)	Enzyme activity, µmol/ml/3min	Inhibition %	Recovery %
Nil	6.05 ± 0.50	0.00	100
$4.7x10^{-3}$	2.95 ± 0.16	51.24	48.76
$4.7x10^{-4}$	3.17 ± 0.21	47.61	52.39
$4.7x10^{-5}$	3.48 ± 0.17	42.48	57.52
$4.7x10^{-6}$	3.78 ± 0.13	37.52	62.48
$4.7x10^{-7}$	3.91 ± 0.28	35.38	64.62
$4.7x10^{-8}$	4.15 ± 0.31	31.41	68.59

The inhibition effect of this compound may be attributed to the similar structure as the structure of Choline ester that's because of containing several (C=O) groups, and then (OH) group of serine (Ser-CH_2-OH) will attack (C=O) group of the compound instead of attacking the (C=O) group of the enzyme, which will cause inhibitory effect of enzyme mechanism.

3.2. Kinetic of Inhibition

Type of inhibition was determined by Lineweaver-Burk plot, Figure (1) which indicate that the inhibition proceeds as competitive inhibition due to change in K_m value while V_{max} remained constant. The competitive inhibition could be represented as follows (24, 25):



$K_{mapp.}$ Was calculated from the point of intercept with 1/[S] axis, where K_i was calculated from $K_{mapp.}$ (26)According to eq. (2)

$$\frac{1}{V} = \frac{Km}{Vmax}\left[1 + \frac{I}{Ki}\right]\frac{1}{[S]} + \frac{1}{Vmax}\left[1 + \frac{I}{Ki}\right] \dots\dots\dots (1)$$

$$Kmapp = Km\left[1 + \frac{I}{Ki}\right] \dots\dots\dots (2)$$

Table (2): Kinetic properties of (AChE) using Lineweaver-Burk plot.

Conc. of Inhib	V_{max} (µmol/ml/3min)	Km (M)	Kmapp (M)
$4.5x10^{-8}$	20	0.0769	$2.1x10^{-4}$

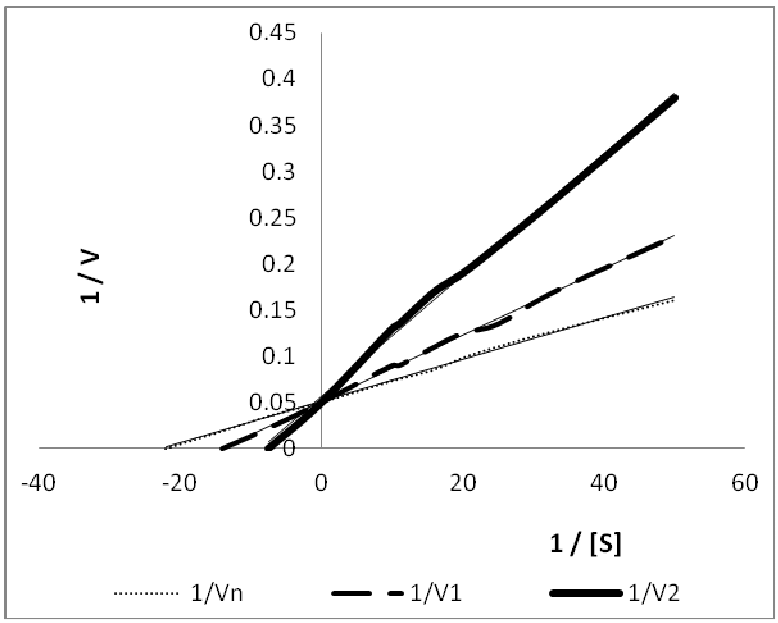


Fig.(1) Line weaver Burk plot determination K_1 and $V_{mapp.}$ Values of inhibition (AChE); (———) normal, (- - -) $4.7x10^{-8}$ M,(▬▬) $4.7x10^{-3}$ M.

Reference:

1. *Juan D, Ding X, Zheng Z, Peng Y. (2002). Synthesis and degradation of intelligent hydrogels containing polyacetal segments. European Polymer Journal. 38(5): 1033-1037.*
2. Matsushima A., Nishimura H. Ashihara Y. Yokota Y. Inada Y. (1980). Modification of E.Coli Aspariginase with 2, 4-Bis (methoxypolyethyleneglycol)-6-chloro-striazine (activated PEG2); Disappearance of Binding Ability Towards Anti-Serum and Retention of Enzymatic Activity. *Chem. Lett* :773-776
3. Ashihara Y, Kono T, Yamazaki S and Inada Y (1978) Modification of E. coli Lasparaginase with polyethylene glycol: disappearance of binding ability to antiasparaginase serum. *Biochem Biophys Res Commun* 83: 385–391
4. Abuchowski A, McCoy JR, Palczuk NC, van Es T, Davis FF.(1977). Effect of covalent attachment of polyethylene glycol on immunogenicity and circulating life of bovine liver catalase. *J Biol Chem.* 252(11):3582–3586.
5. Bariyanga J. (2002) Synthesis, Characterization and Biodegradability of Poly(Ethylene Glycol)-Bound Molecule Platinum Complexcontaining Ferrocenyl Moiety Using MALDI Time-of-Flight. *Mass SpectrometryJournal of Bioactive and Compatible Polymers.* 17: 37-50.
6. Machlin L.J. (1991). Handbook of Vitamins, 2nd ed. Marcel Dekker, Inc.
7. Bossi A. Piletsky S. A. Piletska E. V. Righttl P. G. Turner, A., and Anthony P. F.(2000). An Assay for Ascorbic Acid Based on Polyaniline-Coated Microplates. Anal Chem. 72: p:4296.
8. Yamamoto I. Tai A. Fujinami Y. Sasaki K. Okazaki S.(2002). Synthesis and characterization of a series of novel monoacylated ascorbic acid derivatives, 6-O-acyl-2-O-alpha-D-glucopyranosyl-L-ascorbic acids, as skin antioxidants. *J. Med. Chem*. 45: 462.
9. Ballantyn, B.; and Marrs, T. C. 1992. Clinical and Experimental Technology of Organophosphorous and Curbamate. Butter Worth-Heieman, Oxford, pp: 251-282.
10. Silver A.(1974). The biology of Cholinesterase. American Elsevier Pub. New York.
11. Soreq H. Seidman S. (2001).Acetlcholinesterase-new roles for an old actor. *Nat. Rev. Neuro Sci.* 2:294-302.
12. Shem T. Tai K. Hechman R.H. (2002). Molecular dynamics of acetylcholinesterase. *Ace. Chem. Res.* 35:332-340.
13. Taylor P. Schumacher M. Macphee-Quingley K. et al. (1987). The structure of acetylcholinesterase relationship to its function and cellular disposition. *Trends NeuroSci.* 10:93.
14. Ligand D. Perrier N. Noureddine H. (2008). Old and new questions about Cholinesterase. *Chem. Biol. Interact*. 175 (1-3).

15. Salomon L. L. (1963) Experientia, **19** (12) : 619.
16. Dudley H. and Fleming I. (1995). Spectroscopic methods in organic chemistry. 5th ed. McGraw-Hill companies, London. 24.
17. Mukhlis, A-J. A.; AL-Rawi, M. S., Tomma, J. H. and Al-Dujaili, A. H. (2012). Synthesis And Characterization of New Oxazepines Derived from D-Erythroascorbic Acid. *Ibn Al-Haitham J. for Pure & Appl. Sci.* **25**(2) : 293-307.
18. Carey F.A. (2006). Organic Chemistry. 6th ed. The McGraw-Hill Companies, Inc. New York : 767.
19. WHO.(1978). Spectrophotometric Kit for measuring Cholinesterase activity. WHO/ VBS. 78. 692.
20. Jaffer H.T. Mahmod M.J. Al-Azzawi M.J. (1988). The effect of some 2-mercapto-1,3,4-oxadiazole derivatives on human serum Cholinesterase activity. *J. Biol. Sci. Res.* 19. 793.
21. Gazivoda T. Wittine K. Lovric I. Makuc D. (2006). Synthesis, structural studies, and cytostatic evaluation of 5,6-di-O-modified L-ascorbic acid derivatives. *Carbohydr. Res.* 341 (4) : 433-442.
22. Vogel A. I. (1989). Vogel's Textbook of Practical Organic Chemistry. 5th ed. John Wiley and Sons, Inc. New York :1219.
23. Stepankova K. Komers K. (2008). Cholinesterase and Cholinesterase inhibitors. Department of Biological and Biochemical Science Facutly of Chemical Technology, University of Pardubice. 4 (4):160-171.
24. Al-Mudaffer S. Enzyme inhibition. (1985). Enzyme Kinetics part 1st ed, Baghdad : 393-421.
25. Alexander J. David P. Ballou M. (2010). Biochemistry and Biotechnology.2nd ed.: 257-267.
26. Segal I.H. (1975). Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and steady state Enzyme Systems. John Wiley and Sons, Inc. New York :100-120.

This academic article was published by The International Institute for Science, Technology and Education (IISTE). The IISTE is a pioneer in the Open Access Publishing service based in the U.S. and Europe. The aim of the institute is Accelerating Global Knowledge Sharing.

More information about the publisher can be found in the IISTE's homepage: http://www.iiste.org

CALL FOR PAPERS

The IISTE is currently hosting more than 30 peer-reviewed academic journals and collaborating with academic institutions around the world. There's no deadline for submission. **Prospective authors of IISTE journals can find the submission instruction on the following page:** http://www.iiste.org/Journals/

The IISTE editorial team promises to the review and publish all the qualified submissions in a **fast** manner. All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Printed version of the journals is also available upon request of readers and authors.

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar