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

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Abstract
Polycyclicacetal was prepared from the reaction of PEG with aldehyde derived from Erythro-ascorbic acid (pentulosono-γ-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), 1HNMR, 13CNMR, 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 biomedical 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, organophosphorous (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. 1HNMR spectrum was recorded on Ultra Shield (300 MHz) spectrometer with tetramethyl silane as internal standard. 13CNMR spectrum was recorded on a Varian Mercury plus 100 MHz spectrometer. Electronic spectrum was obtained using a (U.V-Vis) spectrophotometer type CECI 7200 England. Mass spectrum was recorded on IEOLJMS-7 high 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). Rf (0.68) (benzene: methanol, 5:5) (v/v). FTIR (KBr, cm\(^{-1}\)): 3444 (O-H), 3100-3032 (C-H aliph.), 2997 (C-H ali.), 1634 (C=O est.), 1388 (C-O est.), 1278 (C=O est.), 1204 (C=O est.), 1025 (C-O est.).

Synthesis of 2,3-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, 83mmol). The reaction mixture was stirred for 2 hours, then kept in dark place at room temperature for 24 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 x 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) (15 g, 76.5%) as a pale yellow solid (17), m.p (102-104°C). Rf (0.80) (benzene: methanol, 5:5) (v/v). FTIR (KBr, cm\(^{-1}\)): 3028 (C-H ali.), 2983 (C-H ali.), 2939 (C-H ali.), 2843 (OC-H ali.), 1749 (C=O ali.), 1683 (C=O ali.), 1647 (C=C ali.), 1604 (C=C ali.), 1300-1107 (C-O est.), 900-600 δ(C-H) (O.O.P.) (16).

Synthesis of 2,3-O-dianisoyl-1,6-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, 76.7%) as a white crystals, m.p (130-132°C). Rf (0.42). FTIR (KBr, cm\(^{-1}\)): 3444 (O-H), 3008 (C-H ali.), 2972 (C-H ali.), 2843 (OC-H ali.), 1741 (C=O ali.), 1681 (C=O ali.), 1647 (C=C ali.), 1606 (C=C ali.), 1319-1112 (C-O est.), 900-600 δ(C-H) (O.O.P.) (16).

Synthesis of pentulosono-γ-lactone-2,3-enediol (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 x 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). Rf (0.70) (benzene: methanol, 6:4) (v/v). FTIR (KBr, cm\(^{-1}\)): 3040 (C-H ali.), 2983 (C-H ali.), 2843 (OC-H ali.), 2671, 2559 (C-H ali.), 1782 (C=O ali.), 1749 (C=O ali.), 1685 (C=O ali.), 1604 (C=C ali.), 1300-1107 (C-O est.), 900-600 δ(C-H) (O.O.P.). 1H NMR (DMSO δ ppm): 12.5 (s, 1H, CHO), 7.00-7.97 (dd, 8H, aromatic), 3.86 (s, 1H, H4), 3.82 (s, 6H, 2OCH3),(16). 13C NMR (DMSO δ ppm): 167.50 (C=O ali.), 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.). 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 (λmax, nm, CHCl3): 300.

Synthesis of polyethyleneacetal
The aldehyde (pentulosono-δ-lactone-2,3-enediisooate) was dissolved in a mixture of benzene (8ml) and acetic anhydride (50ml) and stirred for 2 hours, then kept in dark place at room temperature for 24 hours. The mixture was poured into ice-water and stirred for 20 minutes, 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). Rf (0.68) (benzene: methanol, 5:5) (v/v). FTIR (KBr, cm\(^{-1}\)): 3240 (O-H), 2993 (C-H ali.), 2908 (C-H ali.), 1751 (C=O ali.), 1662 (C=C), 1431 (-CH3 sym.), 1388 (-CH3 anti.), 1141-900 (C-O), 767 δ(O-H) (O.O.P.) (16).

Synthesis of 2,3,5,6-dianisoyl-5,6-O-isopropylidene-L-ascorbic acid (3)
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 (π → π\(^*\)) and (n → π\(^*\)) 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 \text{alde.}) and the appearance of the band (1279-1068) cm\(^{-1}\) for (-C-O-C\text{ace.}).
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 known 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.

<table>
<thead>
<tr>
<th>Inhibitor Conc. (µ)</th>
<th>Enzyme activity, µmol/ml/3min</th>
<th>Inhibition %</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>6.05 ± 0.50</td>
<td>0.00</td>
<td>100</td>
</tr>
<tr>
<td>4.7x10⁻³</td>
<td>2.95 ± 0.16</td>
<td>51.24</td>
<td>48.76</td>
</tr>
<tr>
<td>4.7x10⁻⁴</td>
<td>3.17 ± 0.21</td>
<td>47.61</td>
<td>52.39</td>
</tr>
<tr>
<td>4.7x10⁻⁵</td>
<td>3.48 ± 0.17</td>
<td>42.48</td>
<td>57.52</td>
</tr>
<tr>
<td>4.7x10⁻⁶</td>
<td>3.78 ± 0.13</td>
<td>37.52</td>
<td>62.48</td>
</tr>
<tr>
<td>4.7x10⁻⁷</td>
<td>3.91 ± 0.28</td>
<td>35.38</td>
<td>64.62</td>
</tr>
<tr>
<td>4.7x10⁻⁸</td>
<td>4.15 ± 0.31</td>
<td>31.41</td>
<td>68.59</td>
</tr>
</tbody>
</table>

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-CH₂-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):

$$
\begin{align*}
E + S &\overset{K_s}{\underset{K_i}{\rightleftharpoons}} ES \overset{K_p}{\rightarrow} E + P
\end{align*}
$$

$K_{mapp}$. Was calculated from the point of intercept with 1/[S] axis, where $K_i$ was calculated from $K_{mapp}$.

(26)According to eq. (2)

$$
\frac{1}{V} = \frac{K_m}{V_{max}} \left[ 1 + \frac{l}{K_i} \right] \left[ \frac{1}{S} \right] + \frac{1}{V_{max}} \left[ 1 + \frac{l}{K_i} \right] ... ... \ (1)
$$

$$
K_{mapp} = K_m \left[ 1 + \frac{l}{K_i} \right] ... ... \ (2)
$$

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

<table>
<thead>
<tr>
<th>Conc. of Inhib</th>
<th>$V_{max}$ (µmol/ml/3min)</th>
<th>$K_m$ (M)</th>
<th>$K_{mapp}$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5x10⁻⁸</td>
<td>20</td>
<td>0.0769</td>
<td>2.1x10⁻⁴</td>
</tr>
</tbody>
</table>
Fig. (1) Line weaver Burk plot determination $K_m$ and $V_{max}$. Values of inhibition (AChE);

$\text{normal}, (-----) 4.7 \times 10^{-8} \text{M}, (-----) 4.7 \times 10^{-7} \text{M}.$

Reference:


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