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Full Length Research Paper

Synthesis and characterization of 1,4-dihydrolutidine from formaldehyde, acetylacetone and plasma albumin

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The 3,5-diacetyl-1, 4-dihydrolutidine adduct was prepared from (acetylacetone), a beta diketone, formaldehyde and plasma albumin and was characterized by measuring its melting point, IR analysis, Uv spectra characteristics as well as the microbial activity. The reagents were mixed in a one pot synthesis and were refluxed for one hour and allowed to stay overnight where a yellow adduct was obtained and was filtered and dried over phosphorus pentaoxide(P_2O_5). The adduct was found to absorbed ultra violet light at three maximum wavelengths, at λ_{max} 250, 255 and 410 nm with an absorption coefficient (ϵ) of1645, 1812 and 1483 L mol^{-1} cm^{-1} respectively . The IR analysis showed the functional groups present in the adduct as follows: The band at 2900.76cm^{-1} was assigned to aldehyde (HC=O) group. The ones at 3850.70 cm^{-1}, 3736.73 70 cm^{-1} and 3617.4770 cm^{-1} were found to be (O-H) stretch for water of hydration and N-H vibrations. The sharp 2356.87 band was found to be due to N-H stretch from amines in the adduct while the bands at 1739.68 and 1547.01 cm^{-1} were attributed to C=O and C-H stretches and the remaining band at 1032.63 cm^{-1} was found to be a C-N stretch due to tertiary amines. The adduct also showed some antimicrobial effect to some micro- organism such *as Bacillus sustilis (12.0mm), Proteum mirabilis (16.0mm) and Canidadalbicans (14.0mm)* as their growth was inhibited when placed around their growth media. It was also found to have no effect on some micro- organisms such as *Escherichia coli and Staphylococcus aureus*when it was placed around their growth media. The melting point analysis showed that the adduct has a melting temperature range of 179.5- 180.4^{o}C. The findings from the study have shown that the prepared adduct is a Schiff base and a protein.

Keywords: AqueousFormaldehyde ,Acetylacetone reagent, Plasma Albumin, synthesis, characterization , 3,5-diacetyl-1, 4- dihydrolutidine adduct.

INTRODUCTION

1,4-Dihydrolutidine adducts are reportedly synthesised from the Hantzsch reaction involving the cyclization of amines, aldehyde specifically formaldehyde and Acetyl acetone a ß- diketones to form diphenyl–1, 4 dihydrolutidine derivative at a temperature of 40^{0}C. This has been observed to share some similarities with the product of the non–enzymatic reaction called Glycation (Povey et al., 2007). There are evidences that under physiological conditions glucose reacts with oxidizing agents non-enzymatically with a wide variety of proteins to form glycated products. Non-enzymatic glycation of proteins has been shown to be a potential problem during their storage in the food and biotech industries (Davis et al., 2001; Smales et al., 2002), and also in human health as a complication of diseases such as diabetes (Al-Abed et al., 1999; Stitt, 2001). The glycation reaction is found to be inhibited in the presence of antioxidants but accelerated in the

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presence of oxidants such as reactive oxygen intermediates free radicals, formaldehyde etc. Glycation is reported to occur via the Maillard reaction in which a reducing sugar reacts with an amino group on a protein, either at the NH_2 terminus or at the e-amino group of lysine residues (Tagami et al., 2000; Yeboah et al., 2004).

Albumin, owing to its abundance in serum, is one of proteins found to undergo glycation and conjugation at multiple sites (Iberg and Fluckiger, 1986). This study under took the syntheses and characterization of a 1,4-dihydrolutidine. The 3,5-diacetyl-1, 4- dihydrolutidine adduct was prepared from a beta diketoneacetylacetone), formaldehyde and plasma albumin and was characterized using melting point, IR, Uv spectra characteristics as well as the microbial activity.

MATERIALS AND METHODS

The Plasma albumin used in this study was previously isolated and purified and fractionated using ethanol based on the methods of Tanaka *et al*., (2001) and Michael, (1962) and was prepared by dissolving 1.0 $gdm^{-3}$of the albumin with distilled water**.** The formaldehyde used was distilled from commercial grade paraformaldehyde, 99% purity while the acetyl acetone reagent used was of analytical grade and no further purification was carried out.

Synthesis of 3,5-diacetyl – 1, 4 –dihydrolutidine derivative

The dihydrolutidine was prepared according to the method described by Hantzsch (Qiong*et al.,* 2007) as follows:
A $20cm^3$Acetyl acetone (0.02mol) solution), $20cm^3$ of 0.0001mol dm^{-3} formaldehyde and $10cm^3$ 0.0001mol plasma albumin were taken in a reflux flask and the mixture was refluxed at room temperature (25^0C) for 1 hr on a water bath. The contents were transferred in a beaker and left to stand overnight after which it was filtered to obtain the yellow dihydrolutidine derivative.The adduct was recrystallize from water and dried over potassium permanganate in a desiccator.

Characterization of the synthesised complex.

The prepared adduct was analysed for its melting point, maximum absorption using uv/visible spectrophotometer and its functional groups using Fourier Transform Infrared spectroscopy as well as its antimicrobial susceptibility test.

Determination of wavelength of maximum absorption (λ max) of dihydrolutidinederivative(0.01) mol dm^{-3}

The **λ**maxof the dihydrolutidine derivative was established using a UV/Vis. Spectrophotometer. The absorbance of the adduct solution were measured at different wavelengths (nm) within the rangeof (199– 400 nm) and at an interval of 5 units and their respective absorbance recorded in Table I. The highest absorbance with the corresponding wave length were then recorded as the **(**λ max) of the adduct. Figure. I

Fourier Transform Infrared Spectroscopy.

The Fourier Transform Infrared spectroscopy (FTIR) analysis of the adduct was carried out on a Nicolet IR spectrometer (IR 100) in the range of 400.75 - $400cm^{-1}$ at 95.586 runs per minute in a KBr pellet mulling agent. The chart was then plotted on an IBM computer using win FIRST plot composer software. The percentage transmittance bands and intensities were read from the chart. Figure 2.

Melting point Analysis

Some quantity of the sample were (0.2g) taken in a capillary tube that was previously sealed at one end. The capillary tube was inserted into a 9100 BI Barmstead electro thermal machine and was turned on. The sample tube was observed through the magnifying lens on the machine over the automated temperature ranges on the machine. The temperatures at which the sample in the capillary tube began to melt and that it finally melted was observed and recorded. Triplicate readings were taken and the results recorded. Table II

Antimicrobial screening test

Antimicrobial screening of the adduct was determined using agar well diffusion method. The organisms collected were cultured into prepared normal saline and was incubated for 30 minutes at 37 ^{0}C. The concentration of each of the organisms was increased to form a turbidity that marched with 0.5 Mcfarlan's standard by visual comparison at which it is assumed that the number of cell is 1.5x10 cfu/mL. The cell suspensions were seeded into the plate of nutrient agar. Wells were bored into the plate of seeded micro-organism using sterile cork borer of 6mm in diameter. At 10 mg/mL of the adduct was constitutedin distilled. This concentration was introduced into each of the wells and was allowed to stand for 30 minutes at 40^0C for

Table I. wavelength of maximum absorption of dihydrolutidine

λ (nm)	**198**	**199**	**200**	**220**	**225**	**230**
Absorbance	**1**	**1**	**1**	**1.405**	**1.773**	**1.457**
Λ(nm)	235	240	245	250	255	260
Absorbance	0.633	1.034	1.306	1.645	1.812	1.478
λ(nm)	265	270	275	280	285	290
Absorbance	1.145	0.880	0.869	0.860	0.713	0.486
λ(nm)	295	300	305	340	345	350
Absorbance	0.164	-0.029	-0.024	-0.006	0.026	0.117
λ(nm)	355	360	365	370	375	380
Absorbance	0.155	0.171	0.342	0.560	0.669	0.779
λ(nm)	385	390	395	400	405	410
Absorbance	0.893	0.971	1.330	1.420	1.449	1.483
λ(nm)	415	420	425	430	435	440
Absorbance	1.400	1.239	1.120	1.017	0.857	0.655

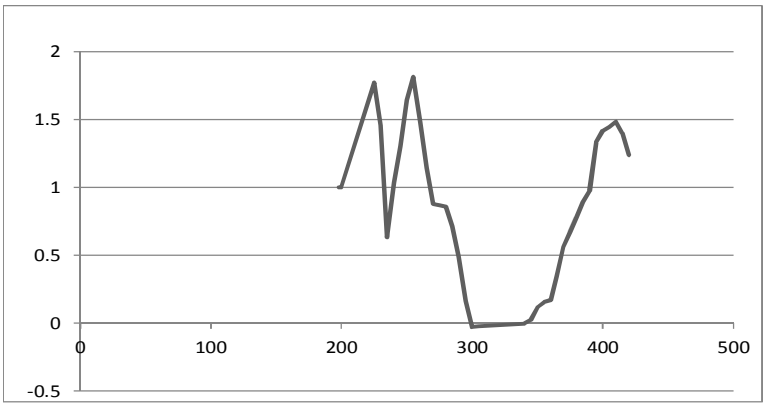

Figure1. The three wavelengths of maximum absorptions λ_{max}

Table II. Results of Melting pointAnalysis

Initial melting temperature of sample ^{0}C		**Final melting temperature ^{0}C**
1st run	- 179.5	180.2
2nd run-180.0		180.4
3rd run	- 180.0	180.5
Sample melting temperature range = 179.83 - 180.4^{0}C		

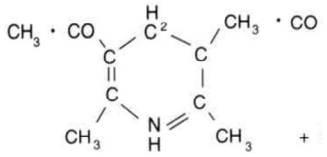

Figure 2. Reaction scheme for the dihydrolutidine synthesis (correct the spelling of dihydrolutidine on the pdf format please).

Table III. Results of antimicrobial screening/susceptibility

Test Organism	Zone of inhibition (mm)
Bacillus sustilis	12.0 mm
Proteum mirabilis	16.0mm
Canidadalbicans	14.0mm
Escherichia coli	0.00mm
Staphylococcus aureus	0.00mm

proper diffusion of the adduct. All the plates were finally incubated at 37^0C for 24 hours and the results were recorded in zones of inhibition in millimetres. Table III.

RESULTS AND DISCUSSIONS.

The synthesised adduct was found to absorbed the ultra violet light strongly at three wavelengths (λ_{max}) 250, 255 and 410 nm with an absorption coefficients (ϵ) of 1645, 1812 and 1483L mol^{-1} $cm^{-}1$respectively. The IR analysisshowed the functional groups present in the adduct as follows: The band at 2948.85 cm^{-1} was assigned to aldehyde (HC=O) group. The ones at 3850.5cm^{-1} 3736.73 and 3617.47were found to be (O-H) stretch for water of hydration and N-H stretches. The sharp 2356.87 band was found to be due to N-H stretch from amines in the adduct while the bands at 1739.68, 1547.01 cm^{-1} and 1032.63 cm^{-1} were attributed to C=O and C-H stretch as well as C-N stretch due to tertiary amines respectively. The adduct also showed some antimicrobial effect to some micro-organism such *as Bacillus sustilis (12.0mm), Proteum mirabilis(16.0mm) and Canidadalbicans (14.0mm)* as their growth was inhibited when placed around their growth media. It was also found to have no effect on some micro- organisms such as *Escherichia coli and Staphylococcus aureus*when it was placed around their growth media. The melting point analysis showed that then adduct has a melting temperature range of 179.5-180.4^{o}C.

CONCLUSION

The findings from the study have shown that the prepared adduct is a pure Schiff base, a protein and has antimicrobial activityjust as the in vivo non-enzymatic reaction between reducing sugars and proteins in glacylation (the covalent bonding of blood glucose to the red blood cells).

These type of substances like lectins, a specialized proteins are found to be non-immunoglobulin in nature and capable of specific recognition as well as reversible binding to carbohydrate moieties of complex glycol conjugates without altering the covalent structure of any of the recognized glycosyl ligands (Davin, 1991).

Rajbar (1968) reported that human blood proteins like hemoglobin and serum albumin undergo slow non-enzymatic Glycation reaction, mainly by forming schiff bases between ϵ- amino groups of lysine and sometimes arginine and glucose molecules in blood resulting to glycol albumin. Elevated glycol albumin has been reported in diabetes mellitus (Iberg, etal. 1986). The Glycation reaction is found to be inhibited in the presence of antioxidants but accelerated in the presence of reactive oxygen intermediates, free radicals etc. Glycation is found to result in the formation of Advance Glycosylation end – products (AGE), which result to abnormal biological effects.

Thus the mechanism of reaction of the prepared adduct may be said to be by covalent binding. This adduct can therefore bind to sugar moieties in cell walls or membranes and thereby change the physiology of

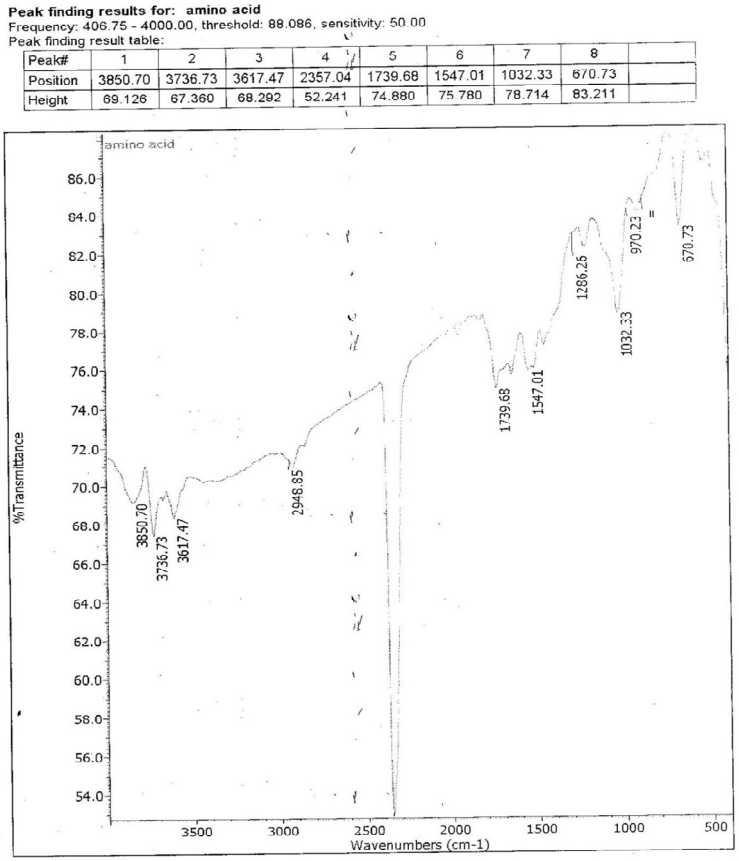

Figure 3. The percentage transmittance bands

the membrane to cause agglutination mitosis, or other biochemical changes in the cell (Swanson, 2010). The adduct may also undertake the non-enzymatic Glycation which might lead to human health complications and diseases such as diabetes (Al-Abed et al., 1999; Stitt, 2001). Glycation is reported to occur via the Maillard reaction in which a reducing sugar reacts with an amino group on a protein, either at the NH_2 terminus or at the e-amino group of lysine residues (Tagami et al., 2000; Yeboah et al., 2004). Albumin, owing to its abundance in serum, is one of proteins found to undergo glycation and conjugation at multiple sites (Iberg and Fluckiger, 1986)

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