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SYNTHESIS AND CHARACTERIZATION OF 3ß-HYDROXYCHOLEST-5-ENE HEMISUCCINATE, A STEROID COMPOUND

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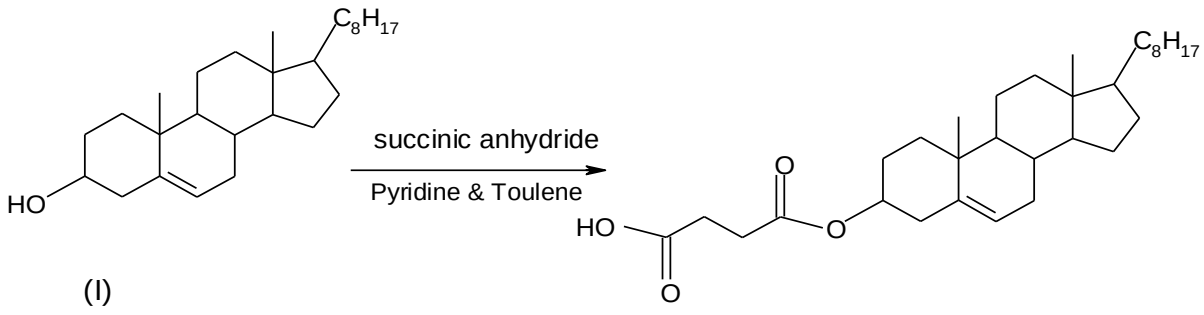
ABSTRACT

Steroids are essential group of natural compounds occurring in living organisms with broad array of biological activities. Such compounds are the fundamental class of biologically signaling molecules which are less toxic and very much bio-available in the living system. This research aimed to synthesize 3ß-Hydroxycholest-5-ene hemisuccinate, a steroid compound and characterize it using spectral studies such as FTIR, ^{1}HNMR and ^{13}CNMR. The method for the synthesis involved esterification of cholesterol with succinic anhydride. The synthesized compound was confirmed by spectrometric studies and found to have complicated kinetic feature as it contained polycyclic rinds. The 3ß-Hydroxycholest-5-ene hemisuccinate may show a good result towards antibacterial activities that should be of medicinal importance.

KEYWORDS: Characterization, Hemisuccinate, Steroid, Synthesis.



(I) (II)

Scheme-1

INTRODUCTION

Steroids are among a large class of organic compounds which occur naturally in plants and animals. They are characterized as having seventeen carbon atoms arranged in the form of 1,2- cyclopentanoperhydrophenanthrane nucleus in their structure which may particularly be reduced or modified. Steroids include compounds with different structures with a variety of compounds which are vital to life, such as cholesterol, bile acids, vitamin-D, sex hormones,

corticoid hormones, cardiac glycones and antibiotics.

Because of their academic and pharmacological importance, a staggering number of steroids have been prepared in laboratory in addition to naturally occurring ones [1]. Steroids are often hormones which human body made naturally. They help the organs, tissues and cells. Many steroids are signaling molecules that activate steroids hormone receptors, while certain steroids (such as cholesterol) are key components of cell membranes that modify membrane fluidity. Animals, plants, and fungus all contain hundreds of steroids such as in cells, lanosterols or cycloaretenol (plants) [2].

A range of tissues, most notably the adrenal gland and gonads, produce steroid hormones and derivatives. Cholesterol is produced in the cell from acetate. Cholesterol ester stores in intracellular lipid droplets, or cholesterol-containing low density lipoprotein uptake. Steroid hormones have 21 carbon atoms or fewer; whereas cholesterol has 27 carbon atoms [3]. The over-secretion of steroid receptors is involved in enhancing cell proliferation in hormone-dependent tumors like breast, uterine, ovarian, prostate, and endometrial cancers. To minimize the growth-stimulating hormonal response of such cancer cells, many techniques have been created. Enzyme inhibitors reduce the biosynthesis of endogenous hormones such as steroid sulfatase inhibitors, aromatase inhibitors (AIs) and 17ß-hydroxysteroid dehydrogenase inhibitors, and ligands compete with endogenous hormones for estrogen receptor (ER) such as antiestrogens. These substances are classed as anticancer steroidal antihormonal/antiproliferative agents [4].

The steroid chemistry has been widely researched, and it can be functionalized at a number of locations along its four ring configurations [5]. Steroids have been employed in a wide range of hybrid materials, including steroid doped liquid crystalline polymers and functionalized nanotubes, as well as gels [6].

The use of steroidal compounds in materials science has been gradually increased while searching for desirable physical properties [7]. An attractive feature of steroidal structures is their uses in crystal engineering, which would take advantage of their tendency to occur in different crystal forms. It also belongs to a group of biologically active polycyclic chemicals that are widely employed for medicinal applications [9]. .Different ring modification investigations of steroidal compounds involving the A-ring and D-ring have shown that inclusion of a heteroatom (N or O) enhances the biological activities of these molecules, proving steroid production.

Antimicrobial, anti-inflammatory, anti-hypotensive, hypocholesterolemic, and diuretic activities have all been demonstrated in such systems [10]. Synthesis of benzylidene

and pyrazoline derivatives of cholesterol and action of pyrazoline derivatives against the HT-29, HCT-15, 502713 cancer cell lines, has been evaluated and the removal of a six-carbon unit from the side chain of cholesterol to generate pregnenolone is the initial step in the manufacture of steroid hormones. Desmolase cleaves the connection between these carbon atoms when the side chain of cholesterol is hydroxylated at C-20 and then at C-22. This remarkable six-electron oxidation consumes three molecules of NADPH and three molecules of O_2. The anterior pituitary gland produces a polypeptide called adrenocorticotropic hormone (ACTH, or corticotropin), which accelerates the conversion of cholesterol into pregnenolone, a precursor to all steroid hormones [11].

Synthesis of steroidal heterocyclic has attracted considerable interest in view of their valuable pharmacological activities. Steroidal azoles have been described as powerful inhibitors of 17a-hydroxylase-C17,20-lyase (CYP17), which can stop androgen production early and hence may be useful in the treatment of prostatic cancer. Also some heterocyclic derivatives have been found to use strong inhibitory effects on 5a-reductases [12].

The aim of research is to synthesize a steroid compound by esterification of cholesterol with succinic anhydride. The objectives of the research are:

1) To characterize synthesized steroid compound (3ß-Hydroxycholest-5-ene hemisuccinate) using FT-IR, ^{13}CNMR and ^{1}HNMR.
2) To identify the kinetic feature of the synthesized compound.

MATERIALS AND METHODS

All reagents and solvents were commercially available and used as received. Melting point was determined on digital auto melting point apparatus. Elemental analysis of the compound was recorded on Perkin Elmer 2400 CHN Elemental Analyzer. The IR spectrum was recorded on KBr pellets with Spectrum Two by Perkin Elmer Spectrometer and values are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were run in $CDCl_3$ on a BrukerAvance II 400 NMR Spectrometer at 400 MHz and 100 MHz respectively. Chemical shifts (d) are reported in ppm relative to the TMS (^{1}H NMR, 400 MHz) and to the solvent signal (^{13}C NMR spectra, 100 MHz) and coupling constants are given in Hz.

Synthesis of 3ß-Hydroxycholest-5-ene hemisuccinate

Mixture of pure cholesterol (200 mg), succinic anhydride (142 mg), 3 mL of pyridine and 10 mL of toluene were gently refluxed for 8 hours and reaction mixture was monitored through TLC for the purity and progress of the reaction. The mixture was cooled at room temperature

and evaporated to a smaller volume which was then diluted with water and extracted with chloroform. The organic phase under reduced pressure was evaporated to dryness; the residue was purified by crystallization from chloroform and methanol which give white solid 195 mg (80%) yield, melting point 175 ^{o}C.

RESULT AND DISCUSSION

The synthesized compound 3ß-hydroxycholest-5-ene hemisuccinate was confirmed from the spectral data.

FT-IR Spectral Analysis

Infrared spectroscopy is used to classify the functional groups and modes of vibration of the compounds. The FT-IR max. cm^{-1}: 2935.30, 1721.13, 1573.63. The synthesized compound shows a characteristic broad band at 2935.30 cm^{-1} which is attributed to O-H stretching vibrations of acid group. Strong peak at 1721.13 cm^{-1} is as a result of C=O stretching of acid group and peak at 1370.42 cm^{-1} and 1446.67 cm^{-1} correspond to symmetric and asymmetric bends of C-H group, respectively while the peak at 1573.63 cm^{-1} indicates the presence of C=C stretching in the aromatic nuclei as shown in Figure 1.

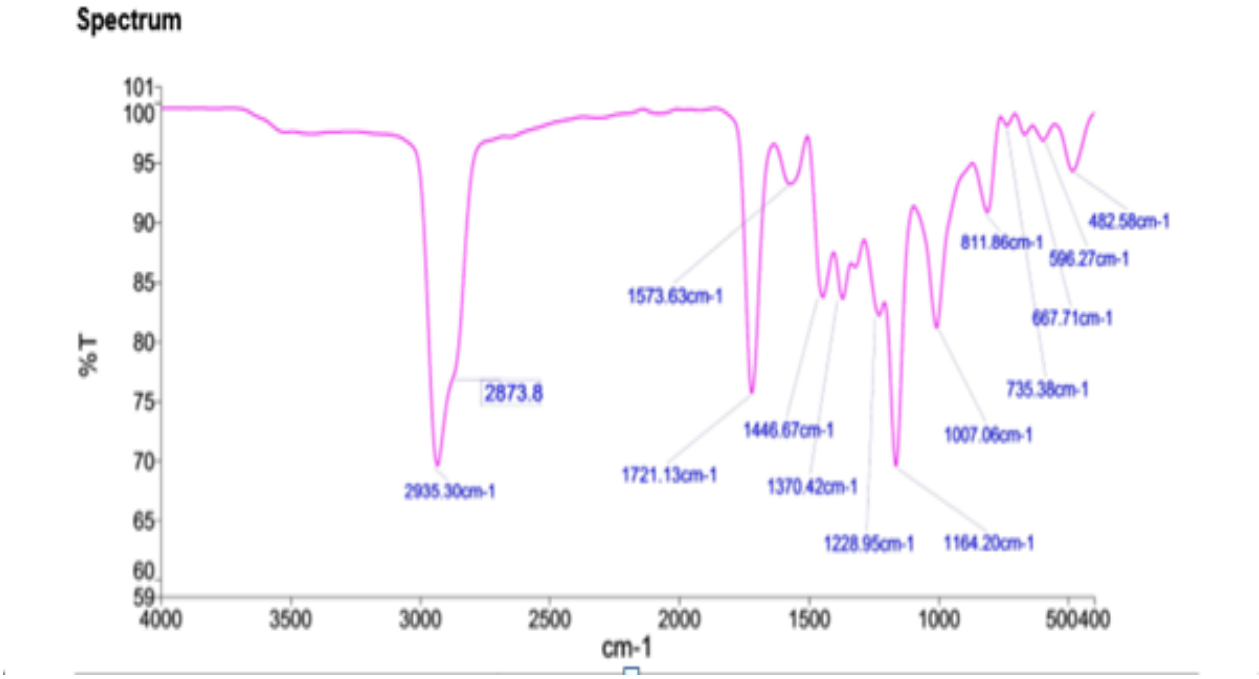


Figure 1: FT-IR spectral Analysis.

1H NMR Spectral Analysis

The 1H NMR spectrum provides useful information about the number of different kinds of protons and also the nature of the immediate environment of each of them. 1H NMR (400 HMz, DMSO-d6) dH: 0.97 (s, 3H), 1.57 (s, 1H), 1.76-1.82 (m, 3H j = 26.9Hz), 1.91 (s, 2H), 2.0 (s, 10H), 2.39-2.40 (d, 2H, j = 10.7Hz), 2.74-2.76 (d, 2H, j = 16.0Hz), 2.89-2.92 (d, 2H, J = 16.0 Hz), 3.21(s, 2H), 3.50 (s, 1H), 5.51 (s, 2H), 6.526 (s, 2H) and 8.17 (broad, 3H) ppm, corresponding to the acidic hydrogen of (COOH), the ^{1}HNMR spectrum of the synthesized compound (Figure 2) showed a strong band at 10.3 corresponding to the acidic hydrogen. It also showed multiple protons with a coupling constant of 26.9Hz, double protons with coupling constant of 10.7 Hz and 16.0 Hz respectively.

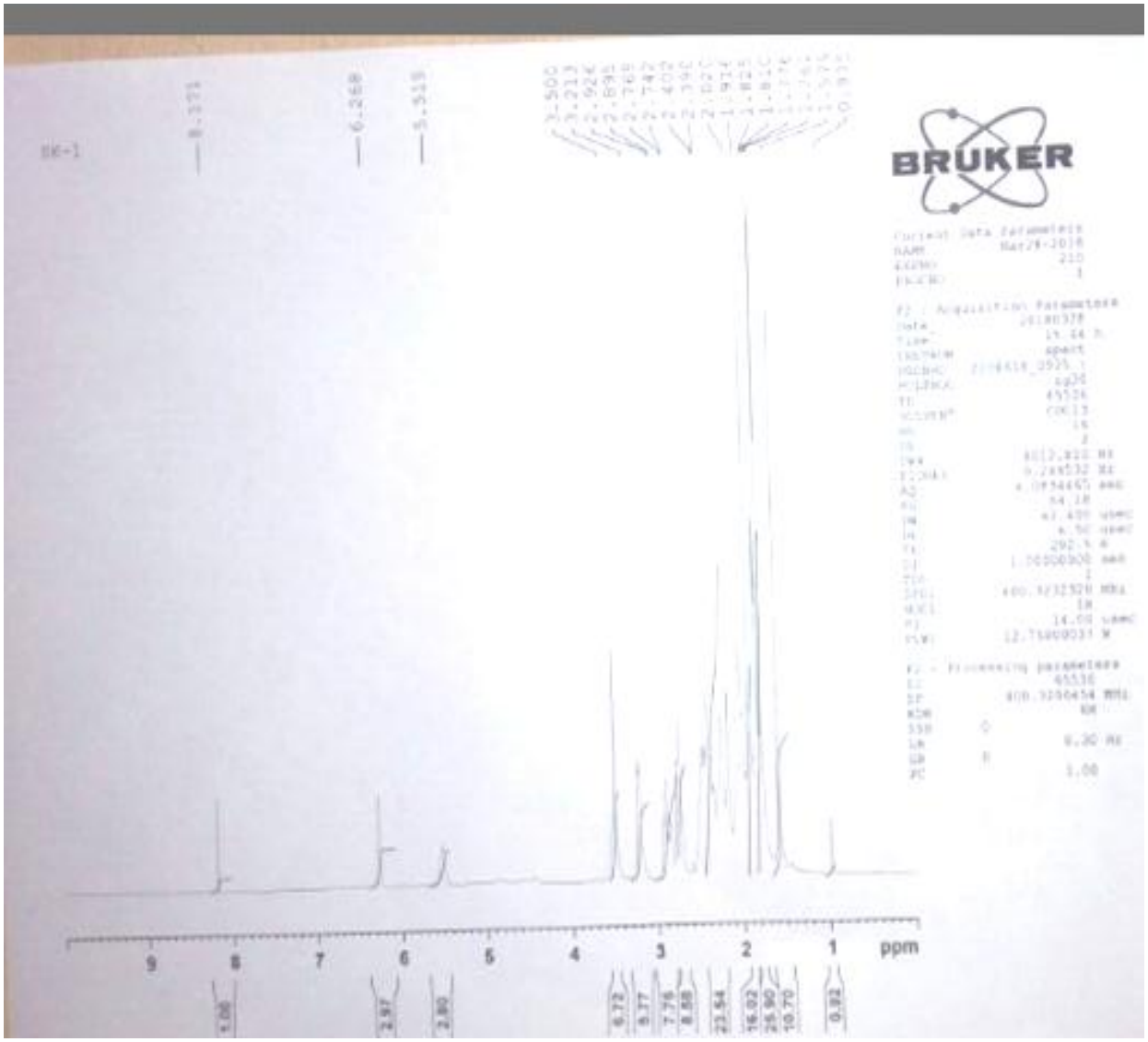


Figure 2: ^{1}H NMR Spectrum

^{13}C NMR Spectral Analysis

13C NMR (100 MHz, DMSO-d6) dC: 27.91 (C-22), 28.20 (C-21), 28.42 (C-19), 29.76 (C-13), 32.02, (C-29), 32.08 (C-17), 35.98 (C-28), 36.02 (C-20), 36.36(C-18), 36.75 (C-5), 37.13 (C-12), 38.24 (C-8), 39.70 (C-34), 39.89 (C-33), 42.49 (C-6), 50.17 (C-10), 56.29 (C-9), 56.85 (C-7), 76.89 (C-27), 77.21 (C- 11), 77.53 (C-26), , 122.8 (C-4), 139.7 (C-16), 171.90 (C-31) ppm. Signals at 122.8 ppm certified the occurrence of double bond at C-5 and C-6, 139.7 ppm shows presence of ester and signal at 171.90 ppm indicates the presence of carboxylic acid groups respectively as shown in Figure 3.

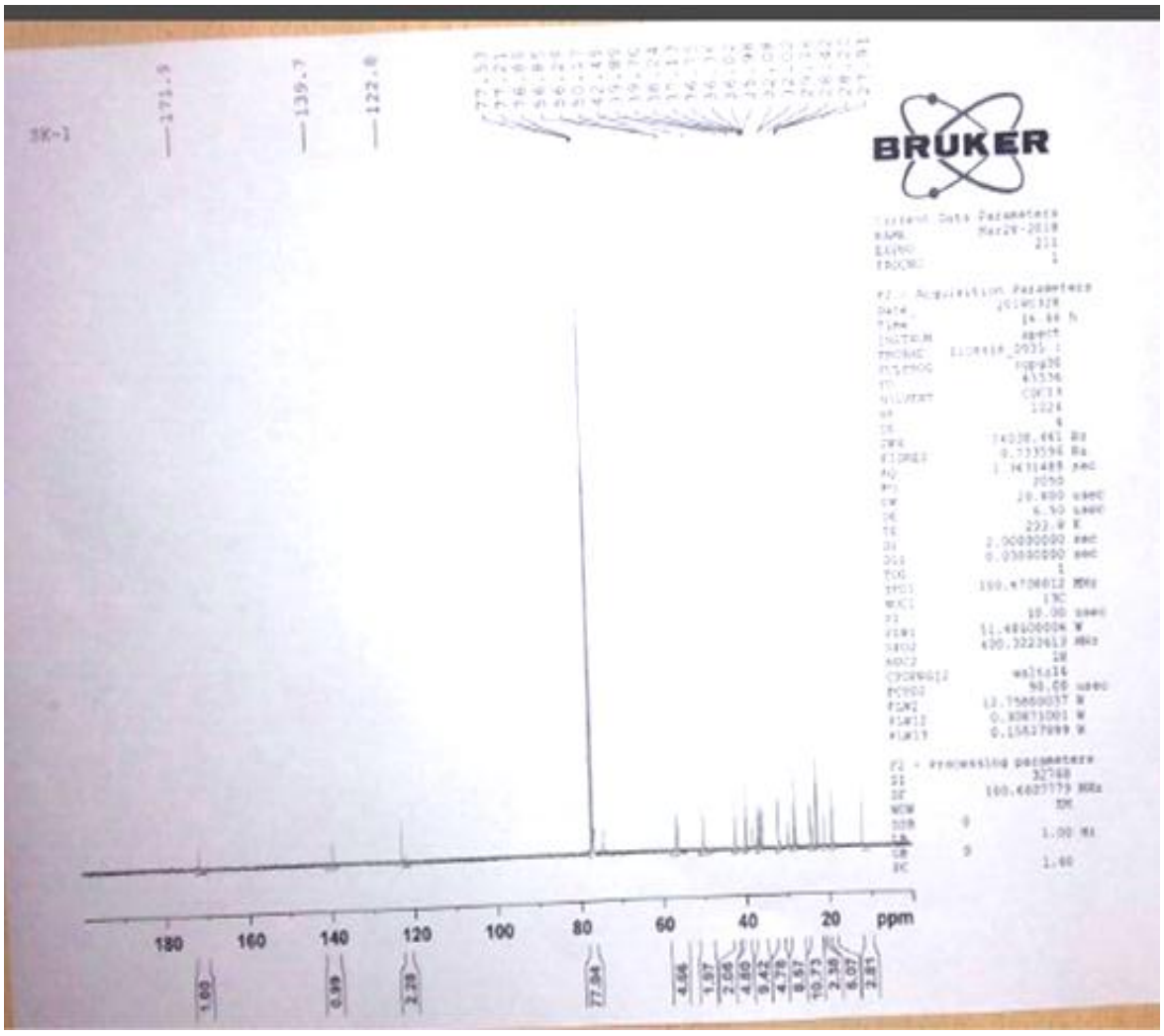


Figure 3: ^{13}C NMR Spectrum.

CONCLUSION

Steroids are important group of natural compounds occurring in the living organisms with broad assortment of biological activities. Such compounds are the fundamental class of biologically signaling molecules, less toxic and highly bio-available in the living system. The 3ß-hydroxycholest-5-ene hemisuccinate was successfully synthesized using cholesterol and succinic anhydride. It was found to contain ester and carboxyl group characteristics. Its molecular structure was characterized successfully and confirmed by elemental analysis, FT-IR, ^{1}HNMR and ^{13}CNMR spectral studies.

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