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Synthesis of 1-Octacosanol from 1, 12 Dodecanediol and Cetyl alcohol

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ABSTRACT

1- Octacosanol($C_{28}H_{57}OH$) plays a major role in pharmaceutical industry and especially in anti hyperlipidemic treatment. However, there exists a need for commercially viable synthetic process for 1- octacosanol from a relatively accessible raw material in a large scale. Herewith we disclose a new synthetic method involving the reaction of two active raw material was devised, namely 1,12 dodecanediol ($C_{12}H_{24}(OH)_2$) and cetyl alcohol ($C_{15}H_{33}OH$). Conversion of 1, 12 dodecanediol into monobromocompound using 48% HBr, followed by hydroxyl protection of the monobromocompound using tetrohydropyran(THP). On a separate reaction, oxidation of cetyl alcohol was carried out to form cetaldehyde using pyridinium chloro chromate. Subsequently, hydroxyl protected bromo compound was coupled with cetaldehyde in presence of Tetra Hydro Furan and lithium. The resultant compound was subjected to dehydration, hydrogenation and de – protection to form 1- octacosanol($C_{28}H_{57}OH$).

Keywords: Octacosanol, Synthesis, Hydrogenation, Oxidation.

1. INTRODUCTION

Drug products of natural origin play a dominant role in pharmaceutical care. This especially is obvious in the case of antitumor drugs, as exemplified by Paclitaxel (Taxol), Vincristine (Oncovin) and various water soluble analogs of Camptothecin (e.g.,Hycamtin). The most efficient method of discovering drugs such as these (i.e.,novel chemical prototypes that may function through unique mechanisms of action) is bioactivity guided fractionation. It is certain that additional natural product drugs, some of which should be useful for the treatment of human beings, remain to be discovered.

Extensive literature survey on 1-Octacosanol, suggest the fact that the compound has undergone array of developmental activities since 19th century. 1- Octacosanol, chemically is a long chain fatty acid alcohols obtained from plant waxes and beeswax has been reported to lower plasma cholesterol in human beings, increases running endurance time and improves biochemical parameters after exhaustion in trained rats [1]. Octacosanol attenuates metabolism of reactive oxygen species which is disrupted in acute liver injury progression, in rats intoxicated with carbon tetrachloride [2]. Further literature survey suggests that 1-Octacosanol is available as a part of policosanol. It is a mixture of few fatty alcohols derived from the waxes of plants such as sugarcane and yams, as well as beeswax [3]. The most prevalent alcohol in policosanol is octacosanol, followed by tricontanol [4]. There is a much lower concentration of several other fatty alcohols like behenyl alcohol, lignoceryl alcohol, ceryl alcohol, 1-heptacosanol, 1-nonacosanol, 1- dotricontanol and geddyl alcohol [5]. In an exhaustive study conducted by Thippeswamy and Salimath [6] two proapoptotic and antiangiogenic compounds from the plant *Tinospora cordifolia* have been identified by activity-guided. Several purification steps and spectral analysis provided with two pure potent proapoptotic and antiangiogenic compounds, T1 and T2. The structure of T2 was found to be $CH_3(CH_2)_{27}OH$, a long chain fatty alcohol - 1-Octacosanol. This compound has already been reported in the literature [7]. However, it has been shown for the first time that Octacosanol possess proapoptic and antiangiogenic activity. The process involved in extracting the desired compound as mentioned by Thippeswamy and Salimath [6], yielded very low levels of octacosanol. Therefore, considering the indicative positive outcome for anticancer activity for octacosanol;

there exists a vital need to carry out the preparation of compound octacosanol in large quantity and assess the comprehensive anticancer activity studies in a systematic form for a compound that was extracted from *Tinospora cordifolia*.

The challenges associated with 1-Octacosanol extraction is that the presence of numerous fatty acid alcohols, hydrocarbon and fatty acids. Most of these chemicals are having close similarities in its chemical structure to that of 1- Octacosanol; hence, the isolation of a pure compound from the rest becomes tedious, expensive and time consuming. Therefore, in this present work, we have attempted to identify a simple and an economically viable method for the extraction and purification of Octacosanol. Our interest was to perform a comprehensive biological activity study for the active 1-Octacosanol and its formulation separately. Attempts have been made to synthesize 1-Octacosanol in a large scale [8] but the same method could not be considered for commercial purpose due to complexities related to hydrogenation process at higher pressure. Therefore, for the commercial procurement of structurally complex 1 - Octacosanol, a novel synthetic method has been developed using 1,12 Dodecanediol and Cetyl alcohol. With the advent of combinatorial chemistry and high throughput screening, even greater progress may now be expected with natural product leads [9].

Figure - 1: The structure of Octacosan-1-ol.

2. EXPERIMENTAL

2.1. Materials

All reagents and solvents used for the synthesis were commercially available and used without further purification.

2.2. Preparation of 1- Octacosanol

2.2.1. Step - I

Bromination of 1,12 – Dodecane diol

In a round bottom flask 1,12-dodecanediol (2.0g,0.01 mol) is taken in benzene (20.0 ml). This is charged with aqueous HBr 48% (1.25mol) with a dean stark condenser .The reaction is refluxed at 70℃ - 80℃ for 6 hours to remove the excess water generated during the reaction. In our process the duration of reaction reduced from 24 h to 6 h this was done in order to prevent the formation of both mono and di bromide compound. After the completion of the reaction (monitored by TLC), the resultant solution is taken out and treated with 10ml of 6NNaOH and then followed by 3M HCl in order to make the final liquid into acidic. To the above reaction mass, further addition of 10 to 15 ml of water. Transfer the content to the separate funnel, followed by the addition of ethyl acetate till the solid materials are fully solubilized, either in aqueous or non layer.

Nonaqueous layer tested for bromocompound of 1,12 dodecane diol through TLC using 10% EA.

10% of EA – For testing the presence of monobromocompound in the crude compound. The resultant compound put through concentration in rotovapour followed by high vaccum evaporation of the compound. Yield of the crude – (2.4g, 95%). The crude taken out for extraction using column chromatography using 10% Ethyl Acetate. The structure and purity of the compound was confirmed by NMR.

2.0g of 1,12-dodecanediol weighed and transferred to a RB flask, 20ml benzene added to the same. This is supplemented with 1.25ml of 48% aqueous HBr. RB mounted on a oil bath with dean stark apparatus and refluxed at 70ºC - 80ºC for 6h to remove excess water (Suk-Ku Kang et al, 1985). The duration of reaction reduced from 24 h to 6 h this was done in order to prevent the formation of both mono and di bromide compound. The aqueous resultant solution is taken out and treated with 10ml of 6NNaOH and then followed by 3M HCl in order to make the final liquid into acidic. To the above reaction mass, further addition of 10 to 15 ml of water. Transfer the content to the separate funnel, followed by the addition of ethyl acetate till the solid material are fully solubilized, either in aqueous or non layer.

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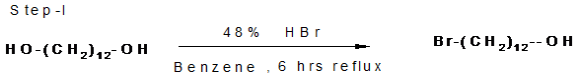


1H-NMR (CDCl3, 400MHz) δ 3.61, t, 2H, -CH_2 - δ 3.39, t, 2H, δ 1.83, q, 2H, δ 1.83, q, 2H, δ 1.53, 3H, -Br-, δ 1.26, m, 16H.

1H-NMR (CDCl3, 400MHz) δ 4.55, s, 1H, δ 3.83, t, 1H, δ 3.72, q, 1H, δ 3.48, m, 1H, δ 3.37, m, 3H, δ 1.80, m, 4H, δ 1.57, m, 6H, δ 1.26, M, 16 H.

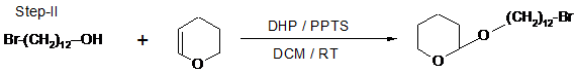
2.2.2. Step - II

In a round bottom flask fitted with calcium chloride guard tube added mono bromo compound 1.65g (0.0062mol) in dichloromehane. It is stirred for 75 minutes to get the clear solution. The reaction mass is charged with 0.782g of PPTS (pyridinum para toluene sulphonate) followed by the addition of DHP (3,4-dihydrohydro pyran) 0.843ml .The reaction mass was stirred for 4 hrs .After the completion of the reaction which was followed by TLC, the reaction mass was extracted with brine solution in DCM. After 1 h reaction, the TLC using 10% EA.

Hydroxyl protection of Mono bromo compound using THP

In a RB flask fitted with calcium chloride guard tube added 1.65 gm of mono bromide compound in dichloro methane. Stirred the reaction mass to dissolve the compound completely in the solvent. Slowly charged 0.782g of PPTS(pyridinum para toluene sulphonate) into the reaction mass followed by the addition of 0.843ml of DHP(3,4-dihydrohydro pyran).The reaction mass was stirred for 4 hrs .After the completion of the reaction which was followed by TLC, the reaction mass was extracted with brine solution in DCM. After 1 h reaction, the TLC using 10% EA

The resulting organic layer was treated with anhydrous sodium sulphate and then concentrated in a rota evaporator to get the crude compound. The material was passed through a silica gel column to get the pure compound. The final product is a clear colorless liquid and the yield is 91%. The structure and purity of the compound was confirmed by NMR and HPLC analysis.



1H-NMR (CDCl3, 400MHz) δ 4.55, s,1H, δ 3.83 t,1H, δ 3.72, q,1H, δ 3.48 , m,1H, δ 3.37, m, 3H, δ 1.80, m, 4H, δ 1.57,m, 6H, δ 1.26, m,16H.

2.2.3. Step - III

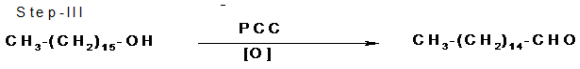
In a RB flask fitted with calcium chloride guard tube added cetyl alcohol 530mg in a 3ml dry DCM (DICHLORO METHANE). The reaction is stirred at room temperature for 10minutes to get the clear solution. To this reaction mass charged PCC (pyridinium chloro chromate) 708mg along with silica gel 710mg .The reaction mass is stirred at room temperature for 3 hours . The reaction completion is confirmed by TLC. After isolating the crude material, it is purified by passing through column to get cetyl aldehyde.

Oxidation of cetyl alcohol to cetaldehyde

In a RB flask fitted with calcium chloride guard tube added cetyl alcohol 530 mg was dissolved in 3.0 ml of dried DCM. The reaction mass was added with 708 mg of PCC (pyridinium chloro chromate) along with 710 mg of silica gel and stirred well. The reaction mass was stirred for 3 hrs and purified by passing through column to get cetyl aldehyde.

The respective product purified using column and also using a small qty of silica gel. The objective of this process is to filter and to receive the pure compound. The resultant compound subjected for concentration. Column chromatography carried out normal elution using 2.5% of EA: Hexane, 5.0% EA;

The pure form of Cetaldehyde is 400mg, yield is 75%w/w.



1H-NMR ($CDCl_3$ + CCl_4, 400 MHz) δ 9.74, s, 1H, , δ 2.40, t, 1H, δ 1.62,t, 1H, δ 1.25, s, 26H, δ 0.87, t , 3H.

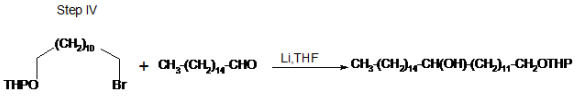
2.2.4. Step - IV

In a round bottomed flask, activated lithium 3.3mg is taken in 10.0ml of dry THF (tetra hydrofuran) under nitrogen atmosphere. In another round bottomed flask protected bromo compound 155 mg from step No.II and celtaldehyde 100mg from step III are stirred together to get clear solution. The mixture is added slowly via additional funnel to the RB flask containing activated lithium in THF under nitrogen atmosphere.

Synthesis of 12 -Hydroxylated protected Octacosanol

In an RB flask fitted with septum under inert atmosphere added 10ml of THF followed by activated Lithium 3.3mg and stirred well. In another RB flask added 155mg of protected bromo compound from step II and 100mg of celtaldehyde from step III, stirred to solubilize the compound. This mixture is transferred to the activated lithium in THF. The reaction was carried out in an inert atmosphere. The respective reaction was carried out in sonicator and the sonication was carried out for the span of 2 h. Checked for the TLC of the 12 hydroxy compound.

Compound obtained is white powder (230mg, 90.1%)



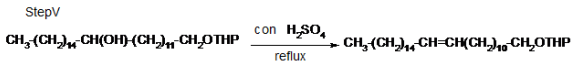
1H- NMR($CDCl_3$ 400 MHz) ; δ 0.8, t, 3H , C1, δ 1.23 to 1.27, q, 48H log chart, δ 3.852, (1H ddd J=8.594, J=3.870, C27), δ H 3.847 (1H ddd J= 8.594, J= 8.060, J=4.430 c27)n , δ 3.4 to 4,H.

2.2.5. Step - V

In a typical procedure a mixture of stage-IV compound 210mg and conc.sulphuric acid 0.25ml is taken in a RB flask. The reaction mass is refluxed for two hours, the final product comes out as white flakes with yield 188mg (89.5%)

Dehydration of Step IV Material

Take in a RB flask 210mg of stage IV material and 0.25ml of Concentrated H2 SO4. Reflux the concentrate for two hours in a RT. The final compound is white flakes and the yield is (188mg, 89.5%)



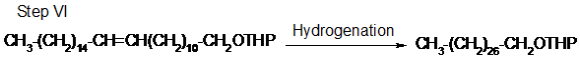
1H- NMR ($CDCl_3$ 400 MHz); δ H 0.8- (3H to C1), δ H 1.23 to 1.27 (48H.q log chart), δ H 3.852 (1H ddd J=8.594, J=3.870, C27), δ H 3.847 (1H ddd J= 8.594, J= 8.060, J=4.430 c27)n , δ H 5 to 5.5

2.2.6. Step - VI

Hydrogenation: A mixture of octacos-13 en 1-THP protected –ol 180mg,(38mmol) and 10% of Pd/C 90mg in THF 40ml is taken into a Parr hydrogenator. The reduction reaction is carried out at 80^{0}C at 35 barr of hydrogen gas pressure for 4 hours.(Giancarlo Cravotto et al., 2010). The progress of the reaction is monitored by TLC at regular intervals. After the completion of the reaction, the reaction mass is filtered on a celite pad and then concentrate the solvent in a rotator evaporator to get the crude hydrogenated product. The pure compound is isolated by purification in column chromatography using solvent hexane: ethyl acetate (19:1) as eluent. Compound was obtained as white powder (165mg, 92% yield).

Hydrogenation

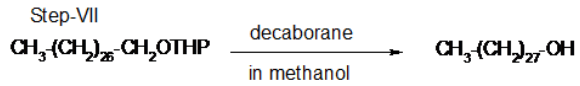
To a solution of octacos-13 en 1-THP protected –ol (180mg,0.38mmol) in THF (40ml), 10% of Pd on charcoal (90mg) was added and pured into a Parr Reactor. The reduction was carried out at 80ºC and 35 bar H2 under stirring for 4h. This is followed by filtration on a celite pad and evaporation under vacuum, a crude product was purified by column chromatography using hexane; ethyl acetate 19:1 elutent . Compound was obtained as white powder (165mg, 92% yield). Analyzed using NMR and the results are as follows



1H - NMR ($CDCl_3$ 400 MHz) δH 0.8- (3H to C1), δ H 1.23 to 1.27 (48H.q log chart), δ H 3.852 (1H ddd J=8.594, J=3.870, C27), δ H 3.847 (1H ddd J= 8.594, J= 8.060, J=4.430 c27)n.

2.2.7. Step - VII

De-protection reaction



Decaborane (1 mol %) was added to a solution of step VI material (1 mmol) in absolute methanol (5mL). The reaction mixture was stirred at r.t.under nitrogen and monitored by TLC using a solution of ethyl acetate and hexane (1:4) (Yeon Joo Jung, 2002). Compound obtained as white powder (110mg, 78%) yield. The compound analyzed for NMR and the results are as follows:

1H-NMR (CDCl3, 400MHz) δ 3.54, t, 2H, J= 6.6 Hz, H-1, δ 1.57, m, 2H, H-2) δ 1.46, m, 5H, H-3, 4, OH), δ 1.29, m, 46H , H-5-27), δ 0.88 ,t, 3H, J=6.3 Hz, H-28). CIMS 411$(M+1)^+$ (C_{28} $H_{58}O$ + H).

3. RESULTS AND DISCUSSION

In this work we have used synthetic method for the preparation of 1-Octacosanol. The starting material diol (1) was treated with hydrobromic acid in benzene and refluxed for 6hrs to get mono - bromo substituted product (2). The resultant product was further subjected to THP protection to get compound (3). Paralelly cetyl aldehyde (5) was prepared from cetyl alcohol (4) with the help of pyridine chloro chromate (PCC) oxidation. THP protected bromo compound (3) and cetyl aldehyde (5) were allowed to react in the presence of lithium using THF as a solvent at -20^0 C to get THP - protected alcohol (6). This compound was further dehydrated with the help of sulphuric acid to get olefinic THP protected alcohol (7). The yielded product was hydrogenated using10% palladium/ Carbon (pd/C) to get saturated THP - protected product (8).This is finally deprotected to give the desired product 1- octacosanol (9).

The brominated product (2) was confirmed by 1HNMR, by considering the integral value that corresponds to OH group at δ value of 2.0, which corresponds to 1H proton. This was further more supported by mass spectroscopy m/z =266.11. Formation of the product (3) was

confirmed by disappearance of OH proton at 2.0 in NMR spectrum. This was also supported by mass spectrum. The product (5) was confirmed by appearance of peak at δ 9.8 and disappearance of peak at δ 2.0 which corresponds starting material OH. The product (6) was confirmed by disappearance of aldehyde peak at δ 9.8 in NMR spectrum and appearance of OH peak at δ 2.0 in NMR spectrum. This was further supported by mass spectrum. Dehydrogenated product (7) was confirmed by appearance of peak at δ 5.4 in 1HNMR spectrum. Hydrogenated product (8) was confirmed by the disappearance of δ value of 5.4 peak and appearance of extra proton at δ 2.9. The deprotected desired product (9) was confirmed by appearance of OH proton at δ value of 2.0 in 1HNMR spectrum. The yield of the final product was found to be 78% w/w and this can be further developed through refined process in the next phase of the process. Moreover, it is evident that the product was obtained through industrially viable methods.

4. CONCLUSION

From the above experiments and the subsequent results, we can conclude and confirm a synthetic process that is robust and effective in terms of yield and industrial feasibility. Further experiments shall be conducted to assess the improvement in yield and in addition, structural and physico – chemical elucidation through Mass Spectroscopy analysis. Nevertheless, the above results provide ample evidence for the synthetic method for Octacosanol from 1, 12 dodecane diol and Cetyl alcohol.

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