RESEARCH ARTICLE

Synthesis of 2,3-O,O-dibenzyl-6-O-tosyl-L-ascorbic Acid

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ABSTRACT- L-Ascorbic acid derivatives was synthesized on treatment with acetone and acetyl chloride afforded 5,6-acetal of L-ascorbic acid then benzylation of C-2 and C-3 hydroxyl groups of the lactone ring was accomplished using K_2CO_3 and benzyl bromide in DMF, then deblocking of the 5,6-O,O-protected derivative of L-Ascorbic acid with acetic acid and methanol gave 2,3-O,O-dibenzyl-L-Ascorbic acid. Subsequently mono-tosylation at 6 position of 2,3-O, O-dibenzyl-L-Ascorbic acid was carried out by addition of p-toluenetosylchloride (PTSC) in Pyridine and MDC solvent medium gave 2,3-O,O-dibenzyl-6-O-tosyl-L-Ascorbic acid. All the structures were characterized by ^{1}H NMR, ^{13}C NMR and Mass Spectroscopy.

Key-words-5,6-Acetal, Benzylation, Hydrolysis, L-Ascorbic acid, Tosylation

INTRODUCTION

Vitamin C, or L-Ascorbic acid, is a vital nutrient for humans and has many important functions in the body. L-Ascorbic acid is a white, odorless, crystalline powder. It is freely soluble in water and relatively insoluble in organic solvent. Vitamin C exhibits anti-scorbutic properties, since it contributes to the synthesis of collagen, the main constituent of the protein fibers in human tissue, which is important in maintaining healthy skin elasticity and texture, and also helps maintain the integrity of substances of mesechymal origin, such as connective tissue, osteoid tissue and dentin [1-4]. L-Ascorbic acid and its derivatives have been found to possess antitumor and antiviral activities [5-7]. Thus L-Ascorbic acid inhibited apoptosis induced by oxidative stress in HL-60 myeloid leukemia cells [5]. L-Ascorbic acid is highly concentrated in the neurons in the brain, which likely indicates the essential roles in neuronal function and antioxidant protection [8,9]. Recent research on the biochemistry of L-Ascorbic acid has been focused on the transport and accumulation mechanism, by which brain acquires L-Ascorbic acid.

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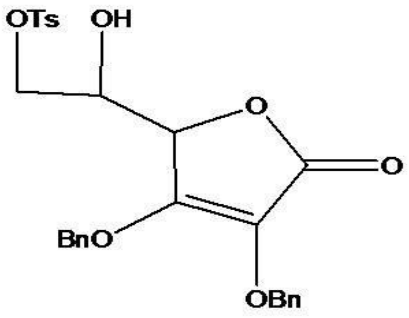
It has been shown that L-Ascorbic acid and its oxidized form, dehydroascorbic acid (DHA), have distinct transport mechanism, mediated by the Na^+-vitamin C transporter SVCT2, and the facilative glucose transporter GLUTI, respectively. [10-13]



(Where Ts-Tosyl Group and Bn-Benzyl Group)

Fig 1: Structure of 2,3-O,O-dibenzyl-6-O-tosyl-L-Ascorbic acid

MATERIALS AND METHODS

Materials

L-Ascorbic Acid was purchased from Sigma-Aldrich, USA and other reagents such as Acetyl chloride, Acetone, benzyl bromide, Potassium carbonate, Dimethylformamide, Acetic acid, Methanol etc were purchased from Finar chemicals Ltd, India and p-toluenesulfonylchloride, Pyridine, Dichloromethane and Diethyl ether were purchased from spectrochem, India. Commercial solvents were used for

work up note during synthesis. All the experiments were performed in Aevum Bio Labs Pvt. Ltd, Hyderabad, India. It took six months time period to overcome this Derivative. All analyses were done in Sapala Organics Pvt. Ltd., India.

Methodology

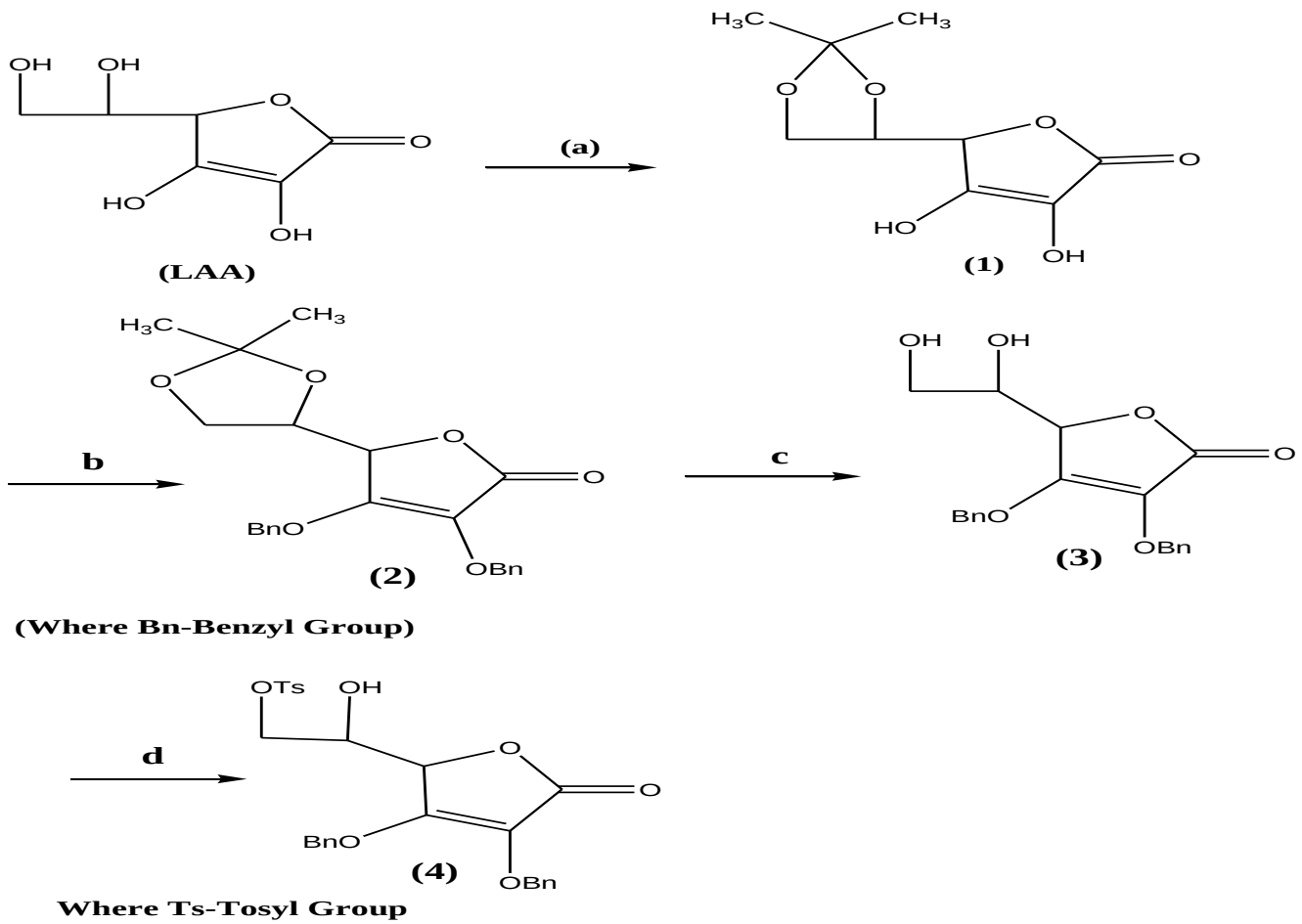
All the Ascorbic acid derivatives were characterized by ^{1}H and ^{13}C NMR and electron impact mass spectra. Melting points of compounds were determined with a Kofler micro hot-stage (Reichert, Wein) were uncorrected. Recoated Merck silica gel 60F-254 plates were used for thin layer chromatography (TLC) and spots were detected under UV light (254 nm). The electron impact mass spectra were recorded with an EXTREL FT MS 2001 instrument with ionizing energy 70 eV. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer, operating at 75.46 MHz for the ^{13}C resonance. The samples were dissolved in DMSO-d_6 or $CDCl_3$ chemical shift values are in ppm, referred to TMS. Additional purification of compound (2) by recrystallization from methanol afforded good purity and acetone was dried over calcium chloride and followed by potassium carbonate in reflux condition. DMF was dried over calcium hydride for above 12hrs in reflux condition and the MDC was dried over calcium chloride and calcium hydride for about 12 hrs. Pyridine was dried by distillation with potassium hydroxide and followed by calcium hydride for couple of Hrs under argon atmosphere. Then Potassium carbonate was dried in oven at $110^{0}C$ for 6 hrs and p-Toluenesulfonylchloride was purified by diethyl ether.

RESULTS AND DISCUSSION

Chemistry

The 5,6-acetal of L-ascorbic acid (1), benzylation compound of the C-2 and C-3 hydroxyl groups (2) and deblocking compound of the 5,6-O,O-protected derivative (3) were synthesized as described previously [14-18]. Synthesis of 2,3-O,O-dibenzyl-6-O-tosyl-L-Ascorbic acid (4) was synthesized previously [14]. In this paper it was synthesized with convenient method with good yield (82%) were outlined in the scheme-1.

SCHEME-1



Reagents and Conditions: (a) Acetone/Acetyl chloride/12 Hrs/RT (b) Benzyl bromide/$K2CO_3$/DMF/12 Hrs/RT (c) Methanol/50% Aq.CH_3COOH/2 Hrs/80-85 ^{0}C (d) Para Toluene Sulfonyl chloride/Pyridine/MDC

Fig 2: Root of synthesis of 2,3-O,O-dibenzyl-6-O-tosyl-L-Ascorbic acid

Experimental Section

Synthesis of 5,6-O-isopropylidene-L-Ascorbic acid (1) was described as priviously[14]. MP 198-202^{0}C, MS m/z 215 (MH^-).

^{13}C NMR (DMSO-d_6) δ: C-1(170.329), C-2(152.527), C-3(118.312), C-4(74.390), C-5(73.575), C-6(64.997), C-7(109.149), CH_3(25.941-25.537), ^{1}H NMR (DMSO-d_6) δ: H-4(4.714-4.708,d,1H), H-5(4.282-4.241,dt,1H), H-6(4.118-4.079,t,1H), H-6(3.901-3.864,t,1H), CH_3(1.255,s,6H), 2-OH(11.302,s,1H), 3-OH(8.489,s,1H).

D_2O Exchange: The peaks at 11.302 and 8.489 were exchanged by D_2O.

The 5,6-O-isopropylidene-2,3-O,O-Dibenzyl-L-Ascorbic Acid (2) was synthesized as described by Von[14].
MP 127-130^{0}C, MS m/z 397 (MH^+).^{13}C NMR ($CDCl_3$) δ:168.976(C-1),121.045(C-2),156.487(C-3),74.530(C-4), 73.658(C-5),65.153(C-6),110.120(C-7),25.802 & 25.562 (CH_3), 73.806 & 73.658(CH_2), 135.839-127.664(C_6H_5).
^{1}H NMR($CDCl_3$) δ:1.409 & 1.366(s,6H,CH_3),4.026-3.988 (dd,2H,H-6),4.272-4.231(dt,1H,H-5),4.537-4.530(d,1H, H-4),5.205-5.063(m,4H,CH_2Ph),7.401-7.195(m,10H,C_6H_5).

Synthesis of 2,3-O,O-Dibenzyl-L-Ascorbic acid (3) was synthesized as described by Von[14]. MP 80.2-83^{0}C, MS m/z 356.9(MH^+). ^{13}C NMR (DMSO-d_6) δ: 169.437(C-1), 158.166(C-2), 120.691(C-3), 74.670(C-4), 68.792(C-5), 61.687(C-6), 136.292-127.738(C_6H_5), 73.633, 72.678 (CH_2Ph). ^{1}H NMR (DMSO-d_6) δ: 4.902(1H,s,H-4), 3.733-3.681(1H,q,H-5), 3.493-3.386(2H,m,H-6), 5.270-5.192 (2H,q,OCH_2), 4.991-4.927(2H,q,OCH_2), 5.165-5.150 (1H,d,5-OH), 4.887-4.873(1H,d,6-OH), 7.429-7.314 (10H,m,C_6H_5). D_2O Exchange: At the region of 5.165-5.150(1H,d,5-OH),4.887- 4.873(1H,d,6-OH) were exchanged by the action of D_2O.

Synthesis of 2,3-O,O-Dibenzyl-6-O-Tosyl-L-Ascorbic acid (4) to a solution of 3 (3.56 g, 0.01 mol) in Dry MDC (7.12 ml) and anhyd. Pyridine (14.24 ml) was stirred at 0^{0}C under argon atmosphere. p-Toluene sulfonylchloride (1.91g, 0.01mol) dissolved in anhyd. MDC (14.24 ml) was added drop wise to this solution. The stirring of the mixture was continued at RT for 24 Hrs. After completion of the reaction, the mixture was diluted with MDC (56.96 ml) and wash with five times of water. Then dried over Na_2SO_4 and evaporated the solvent completely under reduced pressure. Crude product was crystallized from methanol afforded colorless crystals of 4 [4.19, 82% yield, MP 110-111^{0}C and MS m/z 511(MH^+)]. Reaction was monitored by TLC (Mobile phase: N-Hexane: Ethyl acetate 8:2 Ratio, R_f value 0.8),) ^{13}C NMR (DMSO-d_6) δ: 20.97 (CH_3), C-6 (66.520), C-5 (70.653), C-4 (75.510), OCH_2 (74.250, 74.020), C=O (168.967), C-2 (120.930), C-3 (157.038), Ph-Tos (128.380-131.772), Ph-quartC (135.671-136.135), Tos-quartC (131.994-145.167). ^{1}H NMR (DMSO-d_6) δ: 2.411 (CH_3^{tos}, s, 3H), 3.954-3.904 (H-6, q, 2H), 4.171-4.116 (H-5, q, 1H), 4.882 (H-4, s, 1H), 5.789-5.774 (5-OH, d, 1H), 5.244-5.148 (OCH_2, q, 2H), 4.971-4.907 (OCH2, q, 2H), 7.824-7.802, 7.493-7.473 ($H^{Ph\text{-}Tos}$, d, 4H), 7.400-7.290 (H^{Benzyl}, m, 10H). D_2O Exchange: The peak at 5.789-5.774 was exchanged by D_2O.

CONCLUSIONS

In present present work, we described that synthesis of 6-tosylated derivative of 2,3-O,O-dibenzyl-L-Ascorbic acid (3) by using strategy of selective protection and deprotection of 2,3- and 5,6-dihydroxy functional group and subsequent tosylation of 6-hydroxy group. Spectral analysis of 2,3-O,O-Dibenzyl-6-O-Tosyl-L-Ascorbic acid (4) and the structures (1-3) were elucidated by ^{1}H NMR,^{13}C NMR, D_2O Exchange and Mass spectroscopy. The aim of research was to synthesis and biological screening of new nucleoside analogues of L-Ascorbic acid by using the L-Ascorbic derivatives and pyrimidine derivatives with new selective methods, which was performed shortly.

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