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Synthesis and In-Vitro Antioxidant Study Of 5, 6-O, O-Diacetyl-2, 3-O, O-Dibenzyl-L-Ascorbic Acid

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Abstract: 5, 6-0, O-diacetyl-2, 3-0, O-dibenzy l-L-ascorbic acid was synthesized on treatment with acetyl chloride and pyridine in dichloromethane gave white crystalline product with good yield. It is also meant for antioxidant activity. The in-vitro antioxidant study of 5,6-0,0-diacetyl-2,3-0,0-dibenzyl-L-ascorbic acid was carried out by three strains such as Nitric oxide, DPPH and Hydroxyl free radical methods. It was proved to show pronounced in-vitro antioxidant activity. The structure was characterized by ¹H NMR, ¹³C NMR and Mass Spectroscopy.

Key word: 5,6-acetal of L-ascorbic acid, benzylation, hydrolysis, acetylation and in-vitro antioxidant.

Structure



(Where: Ac-Acetyl group and Bn-Benzyl group) Fig 1. Structure of 5, 6-O, O-diacetyl-2,3-O,O-dibenzyl-L-ascorbic acid

I. INTRODUCTION

L-ascorbic Acid is often called Vitamin C, abbreviated as LAA. L-Ascorbic Acid is one of the simplest vitamins and it's a vital nutrient for human beings. L-Ascorbic acid is a white, odorless and crystalline powder. It is freely soluble in water and relatively insoluble in organic solvent. Its derivatives are one of the important bio-molecules which act as anti-oxidant and radical scavenger is widely distributed in aerobic organisms. Thus it protects cellular compounds against oxidative damage by free radicals and oxidants. ^[1] It is a vital nutrient for humans and has many important functions in the body. It plays a central role in the protection of cellular components against oxidative damage by free radicals and oxidants that are involved in the development and exacerbation of a multitude of chronic diseases such as cancer, heart disease, brain dysfunction, aging, rheumatism, inflammation, stroke, emphysema, and AIDS.^[2-18&21]Recently, the chemistry of ascorbic acid has also been exploited to develop strategies for central nervous system drug delivery.^[19] These antioxidant as well as redox and pharmacological benefits of L-ascorbic acid and its derivatives are closely associated with the electron rich C2,C3-enediol moiety of its five-membered lactone ring.^[20]Therefore, the selective modification of its C2- and C3-OH groups is essential for detailed structure-activity studies of L-ascorbic acid. Consequently, our research group was interested in studies^[14-18] involving various ascorbate derivatives as probes for Dopaminemono-oxygenase and Cytochrome b₅₆₁, both of which use ascorbate as a was interested in studies^[14-18] involving various ascorbate derivatives as probes for Dopamine-mono-oxygenase and Cytochromeb₅₆₁, both of which use ascorbate as a source of physiological reductant in catecholamine neurotransmitter biosynthesis. The present study deals with the synthesis and In-vitro antioxidant of 5,6-O,O-diacetyl-2,3-O,O-dibenzl-L-ascorbic acid.



II. MATERIALS AND METHODS

A. 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, pyridine, dichloromethane etc. were purchased from Finar chemicals Ltd, India. Commercial solvents were used for work up note during synthesis. All the analyses were done in sapala organics pvt ltd, India. All experiments were done in aevum biolabs pvt. ltd, India.

B. Methods

All the ascorbic acid derivatives were characterized by ¹H and ¹³C NMR and electron impact mass spectra. Melting points of compounds were determined with a Kofler micro hot-stage (Reichert, Wein) are uncorrected. recoated Merck silica gel 60F-254 plates were used for thin layer chromatography (TLC) an spots were detected under UV light (254 nm). The electron impact mass spectra were recorded with an EXTREL FT MS 2001 instrume with ionizing energy 70 eV. The ¹H and ¹³C NMR spectra were recorded on a Varian Gemini 300 spectrometer, operating at 75.46 MHz for the ¹³C resonance. The samples were dissolved in DMSO-d₆ or CDCl₃ chemical shift values are in ppm, referred to TMS. Column chromatography was performed using silica gel (0.05-0.2nm) Merck; glass column was slurry-packed under gravity. Solvent system used for chromatography was n-hexane:ethylacetate (1:1) in compound 3. Additional purification of compound (2) by recrystallization from methanol afforded good purity, compound (3) was crystallized by n-hexane and di-isopropyl ether (1:1) and compound (4) crystallized by n-hexane. Acetone was dried over calcium chloride and followed by potassium carbonate in reflux condition. DMF was dried over calcium hydride for above 12 hrs in reflux condition and 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 ⁰C for 6 hrs. acetyl chloride was redistilled under calcium chloride.

III. RESULT AND DISCUSSION

A. Compound preparation

5,6-O-isopropylidene-L-ascorbic acid (1),^[23&24] 5,6-O-isopropylidene-2,3-O,O-dibenzyl-L-ascorbic acid (2)^[24] and 2,3-O,O-dibenzyl-L-Ascorbic acid (3)^[24&25] were synthesized in accord with original procedures given in the literature. But the synthesis of 5,6-O,O-diacetyl-2,3-O,O-dibenzyl-L-ascorbic acid was described previously^[26]. But in this paper compound 4 was further modified in a convenient method by the reaction with acetyl chloride and pyridine dichloromethane were outlined in scheme-1.

B. ¹H and ¹³C NMR Studies

The assignment of ¹H and ¹³C spectra were performed on basis of chemical shifts. The ¹H spectra of compound **1-4** exhibit signals of the lactone ring and benzyl groups in positions 2 and 3 of the lactone ring and signals for the protons H-4, H-5 and H-6 of the aliphatic chain of the lactone ring. The methyl group of **1** was absorbed a singlet at 1.255 with six protons. After protection by benzyl group of **1**, the methyl group was absorbed a singlet at 1.366 with six protons in **2** and in **4** the methyl group was absorbed at 2.040 and 1.945 with three protons each peak. The 2-OH and 3-OH of **1** were exchanged by D₂O due to hydrogen bond between O and H atom. In compound **1**, H-4, H-5 and H-6 were doublet, doublet of triplet and triplet respectively. Doublet, doublet of triplet and double doublet in compound 2. In compound 3 singlet, quartet and multiplet, where as doublet, multiplet and double doublet in compound 4. But CH₂Ph was absorbed a multiplet at 5.195-5.087 with four protons in compound 4 and benzyl group at 7.389-7.334 with ten protons in compound 4.

 13 C NMR are given in the experimental section. Generally, the 13C NMR spectra for 1-2 showed nine signals for the lactone ring and for 3-4 showed six and ten signals respectively. In compound 2-4, twelve carbons of benzyl group and two cabons of CH₂Ph were absorbed. All the correlations such as ¹H and ¹³C of compound 1-4 are given in experimental section.

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Reagents and Conditions:(a)acetylchloride/acetone/rt/24hrs (b)Benzylbromide/K₂CO₃/dimethylformamide/rt(c)50% aqueous acetic acid/methanol/85⁰C/2hrs (d)acetylchloride/pyridine/dichloromethane/0⁰C/2hrs.

Fig 2. Root of Synthesis of 5,6-O,O-diacetyl-2,3-O,O-dibenzyl-L-ascorbic acid

C. Experimental

Synthesis of 5,6-O-isopropylidene-L-Ascorbic acid (1): was described previously^[23&24]

MP 198-202⁰C, MS m/z 215 (MH). ¹³C NMR (DMSO-d₆) 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), CH₃(25.941-25.537)

¹H NMR (DMSO-d₆) 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), CH₃(1.255,s,6H), 2-OH(11.302,s,1H), 3-H(8.489,s,1H). D₂O Exchange: The peaks at 11.302 and 8.489 were exchanged by D₂O. Synthesis of 5,6-O-isopropylidene-2,3-O,O-dibenzyl-L-Ascorbic Acid(2): was described previously^[24]. MP 127-130^oC, MS m/z 397 (MH⁺).¹³C NMR (CDCl₃) δ : 168.976(C-),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(CH₃),73.806 &73.658(CH₂),135.839-127.664(C₆H₅). ¹H NMR(CDCl₃) δ : 1.409& 1.366(s,6H,CH₃),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,CH₂Ph),7.401-7.195(m,10H,C₆H₅). Synthesis of 2,3-O,O-dibenzyl-L-Ascorbic acid (3): was described previously^[24&25].

MP 80.2-83⁰C,MS m/z 356.9(MH⁺). ¹³C NMR (DMSO-d₆) 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(C₆H₅), 73.633, 72.678(CH₂Ph). ¹H NMR (DMSO-d₆) 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,OCH₂),4.991-4.927(2H,q,OCH₂),5.165-5.150(1H,d,5-OH),4.887-4.873(1H,d,6-OH),7.429-7.314(10H,m,C₆H₅). D2O 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 D₂O.

Synthesis of 5,6-O,O-diacetyl-2,3-O,O-dibenzyl-L-Ascorbic acid(4): To a cooled (0^{0} C) solutions of 3 (3.56g, 1 mol) in dichloromethane (142 ml) and pyridine (50ml) was added drop wise acetyl chloride (3.14g, 4mol). Reaction mixture was stirred at room temperature for 3hrs and then the reaction was extracted by water (50ml) ten times. Then the organic layer was separated out and was dried over sodium sulfate (Na₂SO₄). The solvent was evaporated out completely under reduced pressure and crystallized by N-Hexane (50ml). Reaction was monitored by TLC (R_f value; 0.8 & mobile phase; 5:5 of n-hexane and ethyl acetate) Product weight 4.0g (91%) MP 56 -58^oC, MS m/z 441(MH⁺).



¹³C NMR (CDCl₃) δ: 168.474(C-1),155.021(C-2),121.177(C-3),73.600(C-4),67.475(C-

 $5), 61.893(C6), 170.153, 169.354(COCH_3), 20.532, 20.359(CH_3), 135.740-134.958, 128.924-127.958(C_6H_5), 73.530(CH_2Ph). \ ^1H \ NMR \ (CDCl_3) \ \delta: 4.808-4.803(1H,d,H-4), 5.360-5.324(1H,m,H-5), 4.348-4.305(1H,dd,H-6), 4.252-4.206(1H,dd,H-6), 2.040(3H,s,CH_3), 1.945(3H,s,CH_3), 5.195-5.087(4H,m,CH_2Ph), 7.389-7.334(10H,m,C_6H_5).$

D. In-Vitro Antioxidant Activity Study

5,6-O,O-diacetyl-2,3-O,O-dibenzyl-L-Ascorbic acid is considered as safe therapeutic agent. It is available in market for various ailments. It is also meant for antioxidant activity. The percentage inhibition of free radicals by in-vitro methods such as DPPH, Nitric oxide and Hydroxyl free radical are carried out. 5,6-O,O-diacetyl-2,3-O,O-dibenzyl-L-Ascorbic acid was proved to show pronounced in-vitro antioxidant activity and its percentage inhibition at 200µg concentration was found to be 67.37 of Nitric oxide method, 98.60 of DPPH method, 35.84 of Hydroxyl free radical method respectively.^[27-29]

Tablet	Conc.						
	10µg	50µg	100µg	200µg	400µg	800µg	1000µg
Nitric oxide	59.45	61.80	63.76	67.37	64.79	60.12	58.85
(% inhibition)							
DPPH	54.62	75.24	93.61	98.60	87.49	84.63	81.65
(% inhibition)							
Hydroxyl radical	24.89	26.57	31.61	35.84	29.54	23.87	18.54
(% inhibition)							

Table-1: In-vitro antioxidant activity of 5,6-O,O-diacetyl-2,3-O,O-dibenzyl-L-ascorbic acid shows maximum percentage inhibition activity at 200 µg concentration.



Fig 3. Nitric oxide (% inhibition) of 5,6-O,O-diacetyl-2,3-O,O-dibenzyl-L-ascorbic acid



Fig 4. DPPH (% inhibition) of 5,6-O,O-diacetyl-2,3-O,O-dibenzyl-L-ascorbic acid





Fig 5. Hydroxyl radical (% inhibition) of 5,6-O,O-diacetyl-2,3-O,O-dibenzyl-L-ascorbic acid



Cumulative Graph of Nitric Oxide, DPPH Scavenging & hydroxyl radical Scavenging

IV. CONCLUSION

The present work describes in-vitro antioxidant study and synthesis of acetylation of 5 & 6 hydroxyl groups of the lactone ring of **3** by using strategy of selective methods. Spectral analysis of 5,6-O,O-diacetyl-2,3-O,O-dibenzyl-L-ascorbic acid (**4**) and the compounds 1-3 were elucidated by ¹H NMR, ¹³C NMR, D₂O Exchange and Mass spectroscopy. The aim of research is the synthesis, biological screening and animal's study of new nucleoside analogues of L-ascorbic acid by using the L-ascorbic derivatives and pyrimidine derivatives with new selective methods, which will be performed shortly

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