THE *C*- AND *O*-BENZYLATION OF L-ASCORBIC ACID

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ABSTRACT

Alkylation of sodium L-ascorbate with benzyl chloride yielded a 2-*C*-benzyl-3-keto-hexulosonic acid lactone and 3-*O*-benzyl-L-ascorbic acid, the yield of each being dependent on reaction conditions. Other products obtained from the alkylations were 2,3-di-*O*-benzyl-L-ascorbic acid and a 1-deoxy-1-*C*-phenyl-2-hexulose. Treatment of 3-*O*-methyl-5,6-*O*-isopropylidene-L-ascorbic acid with benzyl chloride and sodium methoxide yielded the 2-*O*-benzyl derivative.

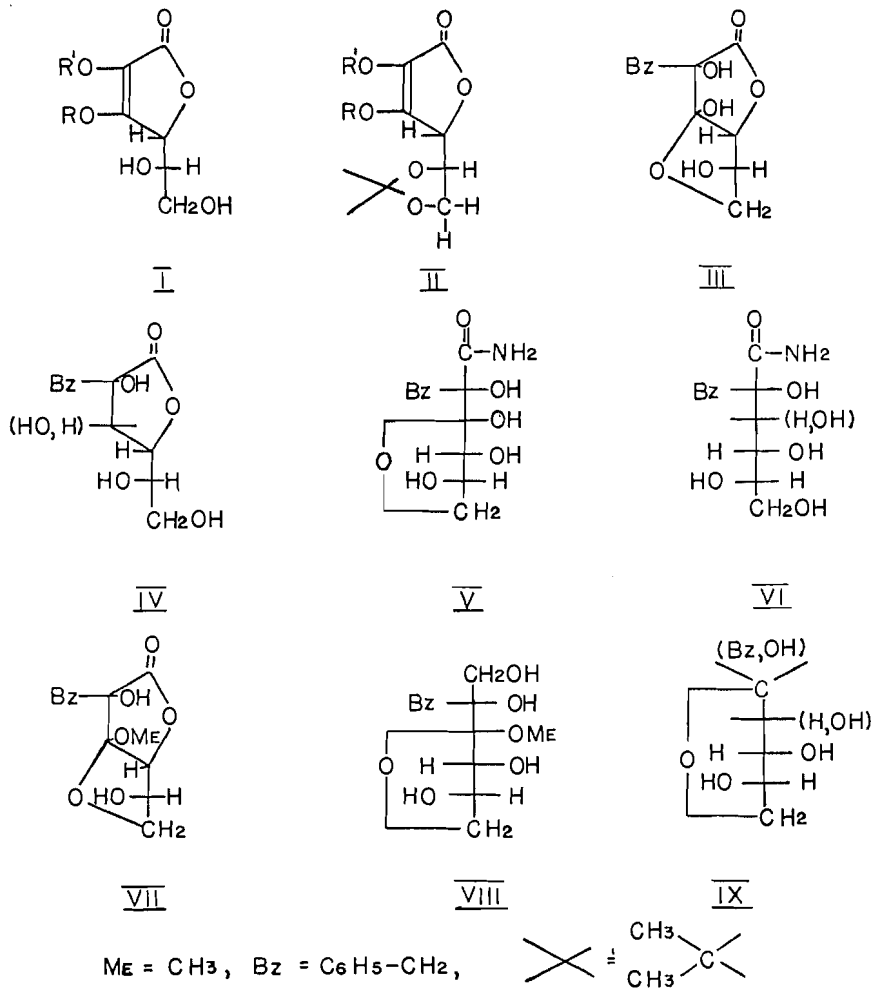
INTRODUCTION

A 2-*O*-methyl-3-hexulose has been prepared from L-ascorbic acid (1). It was argued that preparation of the corresponding *O*-benzyl derivative and subsequent hydrogenolysis of the resultant 2-*O*-benzyl-3-hexulose should yield the parent 3-hexulose.

The reaction of sodium-L-ascorbate with benzyl chloride in aqueous acetone resulted in a mixture of products; the two major components of which were identified as syrupy 3-*O*-benzyl-L-ascorbic acid (I, R = Ph—CH_2, R′ = H) and a crystalline 2-*C*-benzyl-3-keto-hexulosonic acid lactone (III). Derivatives of I, II, and III were prepared.

The lactone (III) did not affect ferric chloride solution and therefore did not contain enolic hydroxyl groups. Aqueous solutions of III were acidic to litmus and gave an instantaneous positive test with Wallenfels reagent (2). The infrared spectrum of III indicated the presence of a lactone and a benzene ring; the absorption of the ascorbate structure was absent. Periodate oxidation revealed the uptake of 5 moles of periodate and the release of 3 moles of titratable acid per mole of lactone. The detection and characterization of phenylacetic acid in the reaction mixture indicated the presence of a *C*-benzyl residue which was attached as a tertiary carbinol. Reduction of III with sodium borohydride provided a neutral product which gave a negative test with Wallenfels reagent. This new crystalline compound showed an absorption corresponding to lactone carbonyl in the infrared and was formulated as a 2-*C*-benzyl-hexonic acid lactone (IV). The amide (VI) of IV was prepared, as well as the amide (V) of III. The optical rotation of each was positive, lending weight to the assignment of the L-*lyxo* configuration to III by Hudson's rules (3). Furthermore the optical rotation of sodium L-ascorbate in acetone–water did not change appreciably over a period of 2 days, indicating that change of configuration at C_4, as experienced by Brenner *et al.*, did not occur (4).

The methyl glycoside (VII) of III was formed in methanolic hydrogen chloride. It did not react with sodium metaperiodate. Molecular models show that only one strain-free glycoside (alpha-L) can be produced. Reaction of the glycoside (VII) in ether with lithium aluminium hydride produced two crystalline compounds, one of which analyzed correctly for the expected alcohol (VIII). Periodate oxidation of VIII caused the formation of 0.6 mole of formaldehyde per mole of VIII; a small amount of acid was also produced. These results indicated that III was 2-*C*-benzyl-3-keto-L-*lyxo*-hexulosonic acid lactone and ruled out the isomeric structure, a 3-*C*-benzyl-2-keto-hexulosonic acid lactone. On treatment with acetic anhydride in pyridine, VIII readily gave a crystalline triacetate in which all of the secondary groups were acetylated.



The sodium salt of III spontaneously decomposed in water giving a crystalline 1-deoxy-1-*C*-phenyl-2-hexulose (IX). This was more easily prepared on a larger scale when L-ascorbic acid and 2 moles of sodium hydroxide per mole were stirred with benzyl chloride. The product was neutral to litmus paper, reduced Fehling's solution, and showed no absorption in the infrared in the interval 1 605 to 2 000 cm^{-1}. Quantitative periodate oxidation of IX resulted in the uptake of 3 moles of periodate per mole and the release of 2 moles of acid per mole. Analysis of the product revealed the presence of glycolaldehyde phenylacetate, both alcohol and acid portions of which were characterized. This substance originates presumably from a 3-hexulose derivative which isomerizes to the 2-hexulose under the influence of base. This wandering of the keto group has been observed to occur in other furano-ketoses and in acyclic ketoses (unpublished results) when a stable ring derivative can be produced.

3-*O*-Benzyl-L-ascorbic acid (I, R = Ph—CH_2, R′ = H) was isolated from the reaction mixture containing 2-*C*-benzyl-3-keto-L-*lyxo*-hexulosonic acid lactone. The separation of I (R = Ph—CH_2, R′ = H) from the amide of III was effected by cellulose column chromatography. On weighing the concentrated fractions of 3-*O*-benzyl-L-ascorbic acid

and V, the amide of III, we found the relative amounts of *C*-benzylation and *O*-benzylation to be in the approximate ratio of 1:1.

A solution of III in chloroform–acetone, after an interval of 3 weeks, crystallized to yield a new lactone which was considered to be an isomorph of III. The isomorph had a lower melting point and a lactone absorption in the infrared (KBr disc) 15 cm^{-1} higher than that of III. Both compounds had similar optical rotations in water, as well as in methanol, and had similar rates of movement on thin layer chromatograms. It was found that the isomorph could be recovered from deuterium oxide by evaporation of the solvent at room temperature. Furthermore, recrystallization of the isomorph from chloroform–acetone solution at 60° gave III only, whereas recrystallization from chloroform–acetone solution at room temperature yielded starting material. The nuclear magnetic resonance (n.m.r.) spectra of each were indistinguishable from one another.

Alkylation reactions in which there is a choice between two reactive sites have been investigated (5–8). It has, in general, been found that *C*-alkylation is favored in media of high hydrogen-bonding power and that *O*-alkylation is favored in media of low hydrogen-bonding power and high dielectric constant such as dimethylsulfoxide and dimethylformamide (9). When dimethylsulfoxide was used as a reaction solvent, a high yield of 3-*O*-benzyl-L-ascorbic acid was obtained.

The crude product so obtained partially crystallized but the crystals proved to be the isomorph of III. Higher substituted derivatives of L-ascorbic acid were removed from the residue by chloroform extraction and 3-*O*-benzyl-L-ascorbic acid (I, R = Ph—Ch_2, R′ = H) was separated from the remaining material by formation of the amide (V) from any III present, followed by chromatography of the mixture on a cellulose column. The weights of *C*-benzylated amide (V) and 3-*O*-benzyl-L-ascorbic acid were in the ratio of 1:3. Syrupy 3-*O*-benzyl-L-ascorbic acid gave a light green color with ferric chloride reagent and showed absorptions at 1 675 and 1 750 cm^{-1} in the infrared. These absorptions were present in all the ascorbic acid derivatives examined. The crystalline isopropylidene derivative (II, R = Ph—CH_2, R′ = H) was readily formed in anhydrous acetone – zinc chloride. 3-*O*-Benzyl-L-ascorbic acid, in excess ethereal diazomethane, gave a syrupy product that did not affect ferric chloride reagent. This was assumed to be 2-*O*-methyl-3-*O*-benzyl-L-ascorbic acid (I, R = Ph—CH_2, R′ = CH_3) and was proved to be such by conversion to crystalline II (R = Ph—CH_2, R′ = CH_3) with anhydrous acetone-zinc chloride reagent.

2-*O*-Benzyl-3-*O*-methyl-5,6-*O*-isopropylidene-L-ascorbic acid (II, R = CH_3, R′ = Ph—CH_2) was prepared from syrupy 3-*O*-methyl-5,6-*O*-isopropylidene-L-ascorbic acid (II, R = CH_3, R′ = H) by treatment of the latter with sodium methoxide followed by benzyl chloride. The melting point and optical rotation of the product obtained (II, R = CH_3, R′ = Ph—CH_2) were found to be significantly different from those of the compound assumed to be 3-*O*-benzyl-2-*O*-methyl-5,6-*O*-isopropylidene-L-ascorbic acid. The infrared spectra were indistinguishable from each other.

EXPERIMENTAL

Paper chromatography was carried out on Whatman No. 1 filter paper by the descending method using the following solvent systems (v/v): (A) butan-1-ol – ethanol – water (3:1:1); (B) butan-1-ol – pyridine – water (10:3:3); (C) ethyl acetate – acetic acid – formic acid – water (18:3:1:4). The sugars were located on paper chromatograms using the following spray reagents: (O) a spray solution of 1% silver nitrate in acetone followed by 2% sodium hydroxide in ethanol (10); (P) a spray solution of 3% *p*-anisidine hydrochloride in butan-1-ol (11) followed by heating the chromatogram in an oven at 115° for 2 min; (Q) a spray solution of 0.01 *M* sodium metaperiodate in water. After 5 min the sugars were located with spray reagent (P). Also used was (R), a spray solution of 2% ninhydrin in butan-1-ol (12). The final detection system, (S) consisted of a glass tank filled with iodine vapor. The chromatograms were suspended in the vapor.

Thin layer chromatography was carried out on glass plates coated with Silica Gel G (Research Specialties Co.). The plate was developed in benzene–isopropanol (2:1, v/v) solvent and then dried in air. Compounds were located as black spots by spraying the plate with concentrated sulfuric acid, followed by heating it at 140–170° in an oven.

Infrared spectra were determined on a Perkin–Elmer Model 21 infrared spectrophotometer. Formaldehyde formation in periodate oxidations was measured by the chromotropic acid method (13). All periodate oxidations were carried out using sodium metaperiodate solutions. Melting points were taken on a Fisher block and are uncorrected. All evaporations were carried out under reduced pressure at 40–60° on a water bath.

C-Benzylation of L-*Ascorbic Acid*

*2-C-Benzyl-3-keto-*L-*lyxo-hexulosonic Acid Lactone (III)*

L-Ascorbic acid (20 g, 0.113 mole) dissolved in water (200 ml) and partially neutralized by potassium hydroxide (5.8 g, 0.105 mole) in water (50 ml) was treated with benzyl chloride (14.4 g, 0.114 mole) in acetone (200 ml). The mixture was shaken mechanically for 60 h at room temperature and concentrated to remove acetone and the residue was washed with petrol (b.p. 60–80°). The residue was then exhaustively extracted with hot ethyl acetate. The extracts were dried (magnesium sulfate), filtered, and the filtrate was concentrated to yield a light-yellow oil. Yields varied from 8.1 g (27% theoretical) to 21.85 g (69% theoretical). The syrup crystallized in one preparation and on recrystallization from chloroform–acetone (8:1) the product had m.p. 156–156.5° with solvent of crystallization eliminated between 70 and 80° and $[\alpha]_D^{23} = +7 \pm 1°$ (*c*, 1.0 in methanol). The crystalline material had R_f 0.76, R_{Rh} 2.50 (spray reagent (O), (P), (Q)) in solvent (A) and R_f 0.82, R_{Rh} 2.04 (spray reagent (O)) in solvent (B). A strong absorption at 1 775 cm^{-1} (lactone carbonyl) was present in the infrared. The material was acidic to litmus; it gave an instantaneous Wallenfels test (2) but did not affect ferric chloride.

Anal. Calcd. for $C_{13}H_{14}O_6$: C, 58.6; H, 5.3. Found: C, 58.5; H, 5.4.

The analysis was performed on solvent-free material, obtained by heating the lactone at 80° for 10 min.

*The Periodate Oxidation of 2-C-Benzyl-3-keto-*L-*lyxo-hexulosonic Acid Lactone (III)*

Oxidations were carried out quantitatively and qualitatively on solvent-free material. In the qualitative oxidation 2-*C*-benzyl-3-keto-L-*lyxo*-hexulosonic acid lactone (200 mg) was dissolved in 0.10 *M* sodium metaperiodate solution (25 ml) in the absence of light. After 4 h the solution was extracted with chloroform (50 ml) and the extracts were evaporated to a gum that crystallized immediately. After recrystallization from petrol (b.p. 60–80°) thin plates of m.p. 74–76° were obtained. The mixed melting point with authentic phenylacetic acid was 75–77°. The infrared spectrum was indistinguishable from that of phenylacetic acid (KBr disc).

In the quantitative oxidation, periodate uptake and acid release were determined from an unbuffered aqueous solution shielded from light. The results are tabulated below.

TABLE I

The periodate oxidation of 2-*C*-benzyl-3-keto-L-*lyxo*-hexulosonic acid lactone

Time (h)	Periodate uptake (moles/mole)	Acid release (moles/mole)
0.25	2.80	1.65
0.75	3.30	2.09
2.33	4.15	2.62
5.25	4.73	2.83
8.25	5.00	3.03
11.00	5.00	3.03

*2-C-Benzyl-3-keto-*L-*lyxo-hexulonamide (V)*

2-*C*-Benzyl-3-keto-L-*lyxo*-hexulosonic acid lactone (1.87 g) in anhydrous methanolic ammonia was kept at −20° for 7 h. Paper chromatography of the solution at the end of this time revealed only one component, moving at R_f 0.62, R_{Rh} 2.00 (solvent (A), spray reagents (Q), (R)) and at R_f 0.74, R_{Rh} 1.50 (solvent (C)). Crystallization occurred when the solution was concentrated to 20 ml and the amide was recrystallized from methanol to give needle-shaped crystals (1.91 g) of m.p. 137–138.5° and $[\alpha]_D^{23} = +7 \pm 1°$ (*c*, 1.0 in methanol).

Anal. Calcd. for $C_{13}H_{17}O_6N \cdot MeOH$: C, 53.3; H, 6.7; N, 4.4. Found: C, 53.3; H, 6.7; N, 4.5.

*2-C-Benzyl-(*L-*idono or* L-*talono)-lactone (IV)*

2-*C*-Benzyl-3-keto-L-*lyxo*-hexulosonic acid lactone (2.69 g, 1.01×10^{-2} mole) in water (100 ml) was added dropwise over a period of 4 h to sodium borohydride (0.404 g, 1.3×10^{-2} mole) in water (100 ml). After it had been stirred for 14 h the solution was still reducing to Benedict's reagent although paper chromatography (solvent (A)) revealed mainly one component of R_f 0.70, R_{Rh} 2.06 with spray reagent

(O). The reaction mixture was treated with acetone (10 ml) and passed through a column of IR-120 (acidic) resin. The effluent was evaporated to a syrup and treated with successive portions of hot methanol (3 × 50 ml) followed by evaporation of the methanol in order to remove boric acid. The syrup so obtained crystallized and recrystallization from chloroform afforded needle-shaped crystals, neutral to litmus and of m.p. 174–175° and $[\alpha]_D^{23} = +39 \pm 2°$ (*c*, 1.0 in methanol).

Anal. Calcd. for a 2-*C*-benzyl-hexonolactone, $C_{13}H_{16}O_6$: C, 58.2; H, 6.0. Found: C, 58.5; H, 6.1. The infrared spectrum was almost identical with the spectrum of the starting material, except for a new absorption at 3 200 cm^{-1} (hydroxyl).

2-C-Benzyl-(L-idonamide or L-talonamide) (VI)

2-*C*-Benzyl-(L-idono or L-talono)-lactone (100 mg) in methanolic ammonia (25 ml) was kept at 0° for 48 h. Paper chromatography (solvent A) revealed the gradual disappearance of starting material and the appearance of a new slower moving component R_f 0.62 and R_{Rh} 1.70 (spray (O), (Q), (R)). The solution was filtered and concentrated to give a light-yellow syrup of $[\alpha]_D^{23} = +24 \pm 2°$ (*c*, 1.0 in methanol).

The Optical Rotation of Sodium L-Ascorbate in Aqueous Acetone (4)

L-Ascorbic acid (1.0 g) was titrated to equivalence (phenolphthalein) with aqueous sodium hydroxide (2.0 *N*) and the solution was diluted with water to a volume of 30 ml. Acetone (30 ml) was then added to bring the total volume to 60 ml. The optical rotation of this solution was measured at various times over a period of 2 days at room temperature.

TABLE II

Optical rotation of sodium L-ascorbate in aqueous acetone

Time (h)	$[\alpha]_D^{23}$
0.16	+132
1.5	+129
24	+127
48	+129

Thin layer chromatography (4) of the product showed the presence only of L-ascorbic acid.

Methyl Glycoside of 2-C-Benzyl-3-keto-L-lyxo-hexulosonic Acid Lactone

A solution of 2-*C*-benzyl-3-keto-L-*lyxo*-hexulosonic acid lactone (1.87 g) in methanolic hydrogen chloride (2% w/v, 100 ml) was heated for 11 h at 55–60°. The solution was neutralized with silver carbonate, filtered, and the filtrate was evaporated to yield a light-yellow syrup that crystallized. The product was recrystallized from hot water to give 0.96 g of needle-shaped crystals of $[\alpha]_D^{23} = +12 \pm 1°$ (*c*, 1.0 in methanol) and m.p. 139–140°.

Anal. Calcd. for $C_{14}H_{16}O_6$: C, 60.0; H, 5.8; OMe, 11.1. Found: C, 59.8; H, 5.6; OMe, 9.0.

The glycoside was not detectable on paper chromatograms (sprays (O), (P), (Q), or in iodine vapor (S)).

Methyl 2-C-Benzyl-L-lyxo-3-hexuloside (VIII)

A solution of the methyl glycoside of 2-*C*-benzyl-3-keto-L-*lyxo*-hexulosonic acid lactone (0.90 g) in anhydrous ether was added slowly to lithium aluminium hydride (1.0 g) in ether (50 ml). Reaction took place immediately with the formation of a precipitate. After 5 min ethyl acetate (5 ml) was added followed by distilled water (50 ml) and the mixture was filtered through Celite. The filtrate was extracted exhaustively with ethyl acetate, to yield, on evaporation of the extracts, a light-yellow oil (0.73 g). This crystallized and recrystallization was effected with ether–petrol b.p. 60–80° (85:15). Two different crops were collected, the first crop (150 mg) having m.p. 144–148° and the second crop (110 mg) having m.p. 115–117°. The product having m.p. 115–117° was recrystallized from ether–petrol b.p. 60–80° (85:15) to give long needles of m.p. 116–118° and $[\alpha]_D^{23°} = -23 \pm 1°$ (*c*, 1.0 in methanol).

Anal. Calcd. for $C_{14}H_{20}O_6$: C, 59.1; H, 7.1. Found: C, 59.4; H, 6.9.

The infrared spectrum showed absorptions at 3 420 and 3 550 cm^{-1} (hydroxyl) and no absorption in the region 1 605 to 1 800 cm^{-1}.

The initial crop of crystals having the melting point 144–148° were recrystallized from water, to give plates of m.p. 149–150° and $[\alpha]_D^{23} = -5 \pm 2°$ (*c*, 1.0 in methanol).

Anal. Calcd. for $C_{14}H_{20}O_6$: C, 59.1; H, 7.1. Found: C, 60.3; H, 6.8.

The infrared spectrum was practically identical with that of the alcohol m.p. 116–118° except for the absence of the absorption at 3 550 cm^{-1} (hydroxyl). The structure of this compound remained unknown.

Periodate Oxidation of Methyl 2-C-Benzyl-L-lyxo-3-hexuloside (VIII)

After 18 h the periodate uptake was determined as 2 moles/mole and formaldehyde release as 0.7 mole/mole. Slow overoxidation resulted in a total periodate consumption of 2.56 moles/mole and an acid realse of 0.63 mole/mole after 60 h.

Methyl 1,4,5-Tri-O-acetyl-2-C-benzyl-L-lyxo-3-hexuloside

The triacetate was prepared by dissolving VIII (100 mg) in pyridine (50 ml) and acetic anhydride (1 ml) and storing the solution at 0° for 10 h. Water (300 ml) was then added and the solution was extracted with chloroform (100 ml). The extract was washed successively with sulfuric acid solution (2 *N*, 2 × 50 ml), saturated sodium bicarbonate solution (50 ml), and water (100 ml) and then dried over calcium sulfate. Concentration of the dried solution afforded a syrup that crystallized immediately. After two recrystallizations from methanol–water the crystals had m.p. 152–154° and $[\alpha]_D^{23} = +14 \pm 1°$ (*c*, 0.62 in chloroform).

Anal. Calcd. for $C_{20}H_{26}O_9$: C, 58.5; H, 6.4. Found: C, 58.3; H, 6.2.

The infrared spectrum (KBr disc) revealed a single hydroxyl absorption at 3 600 cm^{-1}.

Decarboxylation of 2-C-Benzyl-3-keto-L-lyxo-hexulosonic Acid Lactone (III)

The lactone (III) (3.0 g, 1.1 × 10^{-2} mole) in water, was titrated to 77% equivalence (phenolphthalein) with aqueous sodium hydroxide (15 ml, 1.02 *N*). Paper chromatography in solvent (A) revealed mainly one component; R_f 0.19, R_{Rh} 0.60 (spray reagents (O), (P)). The component of R_f 0.19 gradually disappeared over a period of 2 days and was replaced by a substance with R_f 0.62, R_{Rh} 2.15 (solvent (A), spray (O)). After 3 days the solution was evaporated to dryness, extracted with butan-1-ol, and the butan-1-ol solution was filtered, and the solvent evaporated. The heavy oil so obtained was passed through a cellulose column (eluent; butan-1-ol – water, 10:1) and fractions of ca. 6 ml were collected automatically every 15 min. The fractions on which paper chromatographic examination (solvent (A), spray (O)) were shown to contain only the component of R_f 0.62, R_{Rh} 2.15, were combined and evaporated to a light-yellow syrup (1.28 g). The syrup crystallized after 3 weeks and on recrystallization from chloroform–acetone, crystals of m.p. 126–128° were obtained. This was subsequently shown to be a 1-deoxy-1-*C*-phenyl-2-hexulose.

1-Deoxy-1-C-phenyl-L-(lyxo or xylo)-2-hexulose (IX)

L-Ascorbic acid (20 g, 0.113 mole) in water (150 ml) was treated with aqueous potassium hydroxide, (12.8 g, 0.228 mole, 50 ml) benzyl chloride (28 g), and acetone (250 ml) and the system was shaken for 8 h at room temperature. After this interval paper chromatography (solvent A) and spray (O) revealed the presence of only one main component, with R_f 0.62, R_{Rh} 2.15. The solution was evaporated to remove the acetone and washed with petrol (b.p. 60–80°). Exhaustive extraction with hot ethyl acetate and evaporation of the combined extracts provided a syrup, yields of which varied from 11 to 14 g. The syrup crystallized spontaneously only once and further crystallizations were effected by dissolving the syrup in hot acetone (7.1 ml per gram of syrup) and chloroform (1.2 ml per gram of syrup) and seeding the solution. After recrystallization the melting point increased from 115–117° to 127–128° with $[\alpha]_D^{23} = -31 \pm 1°$ (*c*, 1.0 in methanol). The infrared spectrum showed no absorption in the region 1 605 to 1 800 cm^{-1} and was indistinguishable from that of the crystalline product isolated from the decarboxylation of 2-*C*-benzyl-3-keto-L-*lyxo*-hexulosonic acid lactone with alkali.

Anal. Calcd. for $C_{12}H_{16}O_5$: C, 60.0; H, 6.7. Found: C, 59.9; H, 6.8.

Periodate Oxidation of 1-Deoxy-1-C-phenyl-L-(lyxo or xylo)-2-hexulose (IX) and Isolation of Products

The quantitative oxidation on 100 mg of the hexulose (IX) resulted in 3 moles/mole of periodate uptake and 2 moles/mole of acid release after 0.25 h. Little further oxidation occurred after 36 h and no formaldehyde was detected.

In order to isolate the oxidation products the hexulose (0.63 g) was oxidized with sodium metaperiodate solution (0.2 *M*, 7.0 ml) in the absence of light. Chloroform extraction of the reaction mixture after 12 h afforded, after concentration of the extracts, a light-yellow fragrant oil (440 mg) that did not crystallize. The infrared spectrum showed absorptions at 1 740 and 1 720 cm^{-1} (ester, carbonyl). A portion of the syrup (58 mg) was saponified with sodium hydroxide (0.01 *N*, 2.92 ml), acidified with hydrochloric acid, and then extracted with chloroform. The extracts on concentration crystallized immediately and recrystallization from petrol (b.p. 60–80°) afforded plates of m.p. 74–76°. The infrared spectrum was indistinguishable from that of phenylacetic acid (0.8% KBr). A second portion of the syrup (200 mg) was hydrolyzed in aqueous sulfuric acid (0.5 *N*, 10 ml) for 2.5 h, neutralized with sodium hydroxide (phenolphthalein), and evaporated to dryness. Extraction of the salts and organic material with hot acetone yielded, on concentration, a brown gum. A portion of the gum so obtained was dissolved in hot ethanol (60°) containing 2,4-dinitrophenylhydrazine reagent, to give a red–orange product of m.p. 310–320°. The melting point of glycolaldehyde-bis-2,4-dinitrophenylhydrazone prepared from authentic glycolaldehyde was 320–325°. The infrared spectra of the product and of the authentic specimen were indistinguishable from each other.

Isolation of an Isomorph of 2-C-Benzyl-3-keto-L-lyxo-hexulosonic Acid Lactone (III)

The syrupy product from the reaction of L-ascorbic acid with sodium hydroxide and benzyl chloride was dispersed in chloroform–acetone and allowed to stand. After 3 weeks large needle-shaped crystals had formed, with m.p. 142–143° and $[\alpha]_D^{23} = +6 \pm 1°$ (*c*, 0.6 in methanol), $[\alpha]_D^{23} = 13.5 \pm 1°$ (*c*, 2.0 in water) in the unrecrystallized state. The isomorph gave an instantaneous Wallenfels test (2), was acidic to litmus, and revealed an absorption at 1 788 cm^{-1} (lactone) in the infrared and had the same rate of movement as III on thin layer chromatograms. The n.m.r. spectrum, taken with a Varian A-60 spectrometer, D_2O solvent, was found to be identical to that of 2-*C*-benzyl-3-keto-L-*lyxo*-hexulosonic acid lactone. Evaporation of D_2O at room temperature afforded crystals that had m.p. 142–145° with instantaneous

solidification at 145° to a glass that melted at 154–156°. When the isomorph was recrystallized by cooling a solution in hot chloroform–acetone the melting point rose to 154–155° with solvent of crystallization being given off at 70–80°. *Analysis* of the isomorph. Found: C, 58.9; H, 5.5. 2-*C*-Benzyl-3-keto-L-*lyxo*-hexulosonic acid lactone has the following constants. *Analysis*: C, 58.6; H, 5.3; m.p. 156–156.5°, $[\alpha]_D^{23} = +7 \pm 1°$ (*c*, 1.0 in methanol), $[\alpha]_D^{23} = +15.5 \pm 1°$ (*c*, 2.0 in water).

*Isolation of 3-O-Benzyl-*L*-ascorbic Acid (I, R = Ph—CH₂, R′ = H)*

The filtrate after isolation of 2-*C*-benzyl-3-keto-L-*lyxo*-hexulosonic acid lactone gave a light-green color with ferric chloride solution. In a typical separation 5.8 g of crude reaction product gave 0.83 g of crystalline lactone. The filtrate was concentrated, dissolved in methanolic ammonia (100 ml) and left at 0° for 4 days. On concentration of the solution a dark-brown syrup (4.55 g) was recovered. A portion of the syrup (1.77 g) was fractionated on a cellulose column (usual eluent) to yield two main fractions considered to be 3-*O*-benzyl-L-ascorbic acid (800 mg) and 2-*C*-benzyl-3-keto-L-*lyxo*-hexulonamide (580 mg). The infrared spectrum of the former fraction was indistinguishable from that of 3-*O*-benzyl-L-ascorbic acid. The percentage of 3-*O*-benzyl-L-ascorbic acid calculated from the above data was 50%.

O-Benzylation of L-*Ascorbic Acid*

*3-O-Benzyl-*L*-ascorbic Acid (I, R = Ph—CH₂, R′ = H)*

The sodium salt of L-ascorbic acid (17.84 g, 0.09 mole) was dissolved in dimethylsulfoxide (350 ml) containing benzyl chloride (11.43 g, 0.09 mole) and shaken for 1 week at room temperature. Dimethylsulfoxide was removed at 70° *in vacuo* to yield a dark gum. This was dissolved in water (300 ml), washed with petrol (b.p. 60–80°, 100 ml), and exhaustively extracted with hot ethyl acetate. The extracts on evaporation gave a light-brown syrup (19.45 g). Absorptions at 1 690, 1 730, 1 760, and 1 790 cm^{-1} were present in the infrared and indicated the presence of two or more components.

Chloroform extraction of a solution of crude 3-*O*-benzyl-L-ascorbic acid (7.44 g) in water (200 ml) and evaporation of the extracts yielded 1.13 g of dark gum assumed to contain higher substituted derivatives of L-ascorbic acid. The aqueous layer on evaporation gave a syrup of $[\alpha]_D^{23} = +8 \pm 1°$ (*c*, 1.0 in methanol). A portion of the syrup (1.34 g) was dissolved in anhydrous methanolic ammonia and kept at 0° for 48 h. The solution was then concentrated and passed through a cellulose column (usual eluent) to yield two syrupy fractions of total weight 800 mg. The first fraction; R_f 0.77, R_{Rh} 2.63 in solvent (A) (spray (O)) was assumed to be 3-*O*-benzyl-L-ascorbic acid and the second fraction to be 2-*C*-benzyl-3-keto-L-*lyxo*-hexulonamide. 3-*O*-Benzyl-L-ascorbic acid had $[\alpha]_D^{23} = +15 \pm 2°$ (*c*, 1.0 in methanol) and showed absorptions at 1 760 and 1 685 cm^{-1} in the infrared (typical pattern for ascorbate derivatives). There was no shoulder at 1 790 cm^{-1}. The syrup gave a light-green to light-blue color with ferric chloride in methanol and was acidic to litmus. In the above fractions the ratio of 3-*O*-benzyl-L-ascorbic acid to amide was 3:1.

*2,3-Di-O-benzyl-5,6-O-isopropylidene-*L*-ascorbic Acid (II, R = R′ = Ph—CH₂)*

The chloroform extract (1.13 g) (see above) was dispersed in anhydrous acetone (30 ml) containing crushed zinc chloride sticks (2.0 g) and the solution was shaken for 24 h at room temperature. After neutralization with 10% aqueous potassium carbonate (75 ml), extraction of the aqueous solution with diethyl ether, and evaporation of the ether, a light-yellow oil that crystallized immediately was obtained. Recrystallization from ether afforded needle-shaped crystals (0.052 g) of m.p. 125–126° and $[\alpha]_D^{23} = +23 \pm 1°$ (*c*, 1.15 in chloroform). The infrared spectrum showed strong absorptions at 1 755 and 1 675 cm^{-1}. No absorption was present in the region 3 100 to 3 600 cm^{-1}.

Anal. Calcd. for $C_{23}H_{24}O_6$: C, 69.7; H, 6.1. Found: C, 69.4; H, 6.3.

*3-O-Benzyl-5,6-O-isopropylidene-*L*-ascorbic Acid (II, R = Ph—CH₂, R′ = H)*

The isopropylidene derivative was prepared in the usual way (see above) from 3-*O*-benzyl-L-ascorbic acid (1.0 g, $[\alpha]_D^{23} = +8 \pm 1°$). The product crystallized and after recrystallization from diethyl ether – petrol (b.p. 60–80°) afforded needle-shaped crystals (374 mg) of m.p. 109–111° and $[\alpha]_D^{23} = +36 \pm 1°$ (*c*, 1.0 in methanol). The infrared spectrum (KBr disc) showed strong absorptions at 1 750 and 1 690 cm^{-1} with a medium absorption at 3 310 cm^{-1} (enolic hydroxyl).

Anal. Calcd. for $C_{16}H_{18}O_6$: C, 62.7; H, 5.9. Found: C, 62.6; H, 6.0.

*Isolation of the Isomorphous Form of 2-C-Benzyl-3-keto-*L*-lyxo-hexulosonic Acid Lactone (III) from the Experiment with Dimethylsulfoxide*

On standing 4 weeks, the crude reaction product ($[\alpha]_D^{23} = +8 \pm 1°$, (*c*, 1.0 in methanol)) had partially crystallized. The syrupy crystalline mass was dispersed in chloroform – ethyl acetate (4:1) and allowed to crystallize further to yield needles of m.p. 142–144° showing an absorption at 1 790 cm^{-1} (lactone) in the infrared. Except for the lactone peak the infrared spectrum was indistinguishable from that of 2-*C*-benzyl-3-keto-L-*lyxo*-hexulosonic acid lactone.

*3-O-Benzyl-2-O-methyl-*L*-ascorbic Acid (I, R = Ph—CH₂, R′ = CH₃)*

Crude 3-*O*-benzyl-L-ascorbic acid (3.42 g) was dissolved in absolute methanol (25 ml) containing excess ethereal diazomethane and kept at 0° for 2 days. The solution on evaporation yielded a syrup. The syrup was dispersed in chloroform and washed with water. The chloroform layer was dried and evaporated to yield a neutral gum (1.73 g) of $[\alpha]_D^{23} = +21 \pm 2°$ (*c*, 1.0 in methanol). The infrared spectrum of the gum showed strong peaks at 1 758 and 1 672 cm^{-1}.

3-O-Benzyl-2-O-methyl-5,6-O-isopropylidene-L-ascorbic Acid (II, R = Ph—CH_2, R' = CH_3)

The isopropylidene derivative was prepared from 3-*O*-benzyl-2-*O*-methyl-L-ascorbic acid in the usual way. The product was recrystallized from diethyl ether to give needles of m.p. 99.5–100.5° and $[\alpha]_D^{23}$ = +36 ± 1° (*c*, 1.0 in methanol).

Anal. Calcd. for $C_{17}H_{20}O_6$: C, 63.7; H, 6.3. Found: C, 63.9; H, 6.3.

Two strong absorptions at 1 675 and 1 750 cm^{-1} and no hydroxyl absorption were present in the infrared spectrum.

3-O-Methyl-5,6-O-isopropylidene-L-ascorbic Acid (II, R = CH_3, R' = H)

The isopropylidene derivative was prepared from 2.0 g of 3-*O*-methyl-L-ascorbic acid (14) in the usual way except that the product was extracted with ethyl acetate. The yield was 1.32 g of a colorless syrup of $[\alpha]_D^{23}$ = +18 ± 1° (*c*, 1.0 in methanol). Paper chromatography (solvent (A)) revealed one component moving at R_f 0.84, R_{Rh} 2.81 (spray (O), iodine vapor).

2-O-Benzyl-3-O-methyl-5,6-O-isopropylidene-L-ascorbic Acid (II, R = CH_3, R' = Ph—CH_2)

3-*O*-Methyl-5,6-*O*-isopropylidene-L-ascorbic acid (1.32 g) in anhydrous methanol (50 ml) was treated with sodium methoxide (0.7 ml, 4.0 *N*) and the solution was concentrated to a syrup. Benzyl chloride (30 ml) was added and the mixture heated for 1 h to give a dark-brown solution. The reaction system was removed from the bath, treated with water (200 ml), and steam distilled to remove the benzyl chloride. Crystallization commenced after the removal of the benzyl chloride and after recrystallization from ether the product had m.p. 86–87° and $[\alpha]_D^{23}$ = +41 ± 1° (*c*, 1.0 in methanol).

Anal. Calcd. for $C_{17}H_{20}O_6$: C, 63.7; H, 6.3. Found: C, 63.45; H, 6.3.

The infrared spectrum was indistinguishable from that of 3-*O*-benzyl-2-*O*-methyl-5,6-*O*-isopropylidene-L-ascorbic acid.

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