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Ascorbic Acid



Figure 1.1 Albert Szent-Györgyi (b. 16.9.1893–d. 22.10.1986), in 1911, started to study medicine at the University of Budapest. This was followed by his cosmopolitan years in Prague, Berlin, Hamburg, Leiden, Groningen, Cambridge, and Rochester, before accepting a chair at the University of Szeged in 1930. In 1947, he moved to the Marine Biology Laboratories in Woods Hole (MA) in the United States. In 1937, Albert Szent-Györgyi was awarded the Nobel Prize for Medicine for his research on vitamin C. Source: J.W. McGuire, https://commons.wikimedia.org/wiki/File:Albert_Szent-Gy%C3%B6rgyi_cropped.jpg. CC-public domain.

A strongly reducing substance, $C_6H_8O_6$, was isolated from the adrenal glands in 1928 by Szent-Györgyi [1]. This substance was later identified as vitamin C, the essential food constituent, the lack of which leads to scurvy (in French, “scorbut”). Hence, this substance was given the name **ascorbic acid** [2].

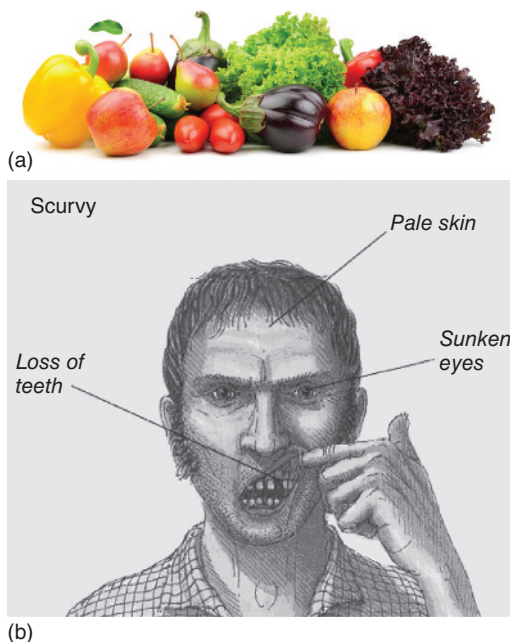
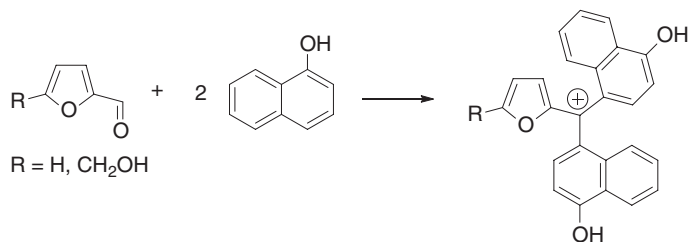


Figure 1.2 (a) Fruits containing vitamin C. (b) Signs of scurvy. Source: (a) Serg64/Shutterstock, www.gettyimages.com (b) Dorling Kindersley Ltd, Gütersloh, Germany. With kind permission of Dorling Kindersley Ltd, Gütersloh, Germany.

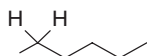
The highly oxygenated nature of this substance indicated a relationship with carbohydrates, and, indeed, ascorbic acid showed a positive *Molisch* test. The molecular formula suggests ascorbic acid to be a dehydrogenated ($-4H$) hexose.

Information Box 1 Molisch Test for Pentoses and Hexoses [3, 4].

Pentoses and hexoses, therefore carbohydrates in general are dehydrated by sulfuric acid to generate furfural or hydroxymethyl-furfural. When this is effected in the presence of a phenol, such as α -naphthol, condensation to give a red or violet colored triphenyl-methane dye is initiated.



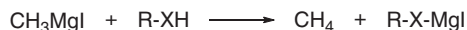
1.1 Preliminary Findings. Upon treatment with chloro(triphenyl)methane, ascorbic acid readily formed a trityl ether [5]. Hence, ascorbic acid contains a primary alcohol function. Upon treatment with HCl, ascorbic acid formed furfural quantitatively [6]. Accordingly, ascorbic acid contains at least five C-atoms in a linear chain.



Ascorbic acid readily formed an acetonide [7]. It should, therefore, be a 1,2- or 1,3-diol. Finally, it was established with a *Zerewitinoff* test that ascorbic acid contains four H-atoms active toward *Grignard* reagent CH_3MgI [8].

Information Box 2 Zerewitinoff Test for Active Hydrogen [9].

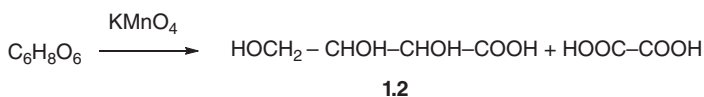
Methyl-Grignard reagents such as CH_3MgI react with all compounds containing a $-\text{OH}$, $-\text{NH}_2$, $-\text{NH}$, $-\text{SH}$, $-\text{C}\equiv\text{CH}$ group under evolution of methane. The methane evolved can be quantified volumetrically to determine the number of $-\text{XH}$ groups in the compound investigated.



As the name implies, ascorbic acid is acidic, with a $\text{p}K_{\text{A}}$ value of 4.1 [5]. Ascorbic acid thus readily yielded a sodium salt $\text{C}_6\text{H}_7\text{O}_6\text{Na}$ [8]. Upon reaction of ascorbic acid with CH_2N_2 , two (acidic) OH groups were methylated to give a dimethoxy compound **1.1** $\text{C}_8\text{H}_{12}\text{O}_6$ [8, 10].

Ascorbic acid as well as its sodium salt gave a strong positive result for the Fe^{3+} color test for enols [8], whereas that of the dimethoxy compound was negative. Therefore, at least one of the acidic H-atoms in ascorbic acid belongs to an enol, and the other one may belong to a second enol or to a carboxylic acid.

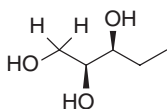
1.2 Cleavage of ascorbic acid into smaller fragments was accomplished by oxidation: upon oxidation with NaOI , 1 equiv. of oxalic acid was obtained [6]. Oxidation of ascorbic acid by KMnO_4 furnished oxalic acid and a 2,3,4-trihydroxybutanoic acid **1.2**; Scheme 1.1 [6, 11].

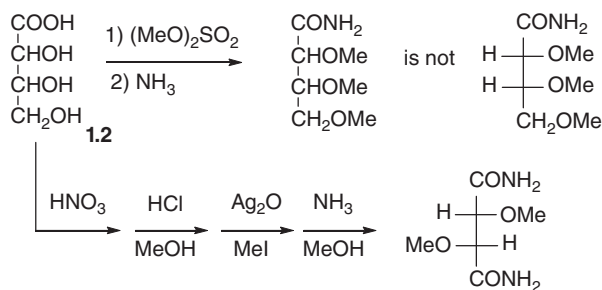


Scheme 1.1

1.3 Since there are two diastereomeric forms of 2,3,4-trihydroxybutanoic acid, the relative configuration of **1.2** was addressed at this point. To this end, compound **1.2** was permethylated with $(\text{MeO})_2\text{SO}_2/\text{KOH}$, and the methyl ester obtained was converted with NH_3 to a crystalline amide (Scheme 1.2).

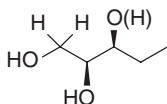
This amide turned out to be different from the known amide of *erythro*-2,3,4-trimethoxy-butanoic acid. Hence, compound **1.2** ought to be the *threo*-diastereomer thereof [11].





Scheme 1.2

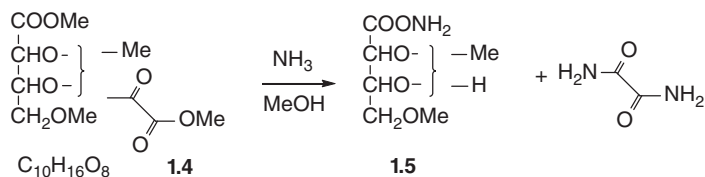
The *threo*-configuration was established by oxidizing compound **1.2** with HNO_3 (Scheme 1.2). This led to a tartaric acid, which was identified after esterification, O-methylation, and amide formation as being the (*R,R*)-tartaric acid. These findings established the following partial structure of ascorbic acid:



1.4 The as-yet-unidentified right-hand part of ascorbic acid contains one more C-atom and three O-atoms including the enol function, identified under Section 1.1. Further evidence was sought by methylating (blocking) all OH-functions followed by cleavage of the $\text{C}=\text{C}$ bond, starting with the dimethoxy compound **1.1** $\text{C}_8\text{H}_{12}\text{O}_6$ described under Section 1.1, which still contained two OH groups. Thus, compound **1.1** was methylated with $\text{MeI}/\text{Ag}_2\text{O}$ to give a tetramethoxy compound $\text{C}_{10}\text{H}_{16}\text{O}_6$ (**1.3**) [11].

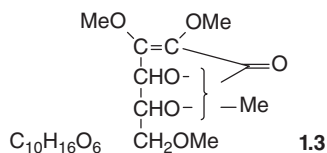
The latter should still contain the enolic $\text{C}=\text{C}$ bond, which could be cleaved with O_3 to give a neutral compound $\text{C}_{10}\text{H}_{16}\text{O}_8$ (**1.4**) (Scheme 1.3). Compound **1.4** retained all 10 C-atoms of its precursor **1.3**. Therefore, the enolic $\text{C}=\text{C}$ bond in the tetramethoxy compound $\text{C}_{10}\text{H}_{16}\text{O}_6$ must have been part of a ring! Ozonolysis of an enol ether gives rise to an ester. To cleave the ester moiety, compound **1.4** was treated with NH_3 , resulting in oxamide and the amide **1.5** of a hydroxy-dimethoxy-butanoic acid [11].

In this transformation, three $\text{CO}-\text{NH}_2$ moieties have been generated. Therefore, **1.4** must have contained three ester functions. Yet, bookkeeping of the atoms

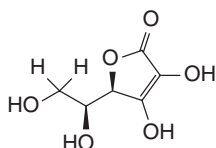


Scheme 1.3

allows for only two methyl esters in compound **1.4**. Hence, the third ester function should be a lactone unit. Accordingly, the precursor tetramethoxy compound $C_{10}H_{16}O_6$, the permethylated ascorbic acid, **1.3**, must have been a lactone and a dimethyl ether of an ene-diol.

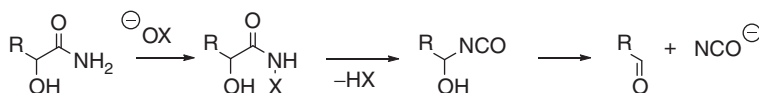


1.5 All that remained at this point was to determine the ring size of the lactone. This could be determined by locating the position of the free OH group in the hydroxydimethoxy-butanamide **1.5**. To this end, compound **1.5** was subjected to *Weerman* degradation by the action of NaOCl [12]. The liberation of cyanate in this process evidenced the presence of an α -hydroxy-carboxamide in **1.5**. Hence, the lactone ring must have been five-membered, and already present in ascorbic acid, the structure of which was thus established as the enol form of 3-keto-L-gulonolactone:



Information Box 3 Weerman Degradation [12].

In the Hofmann degradation a carboxamide $R-CONH_2$ is cleaved to $R-NCO$ and CO_2 . Weerman found that when the residue R contains an α -hydroxy-function, the resulting isocyanate undergoes hydrolysis to cyanate and an aldehyde. This reaction has been applied to the stepwise degradation of carbohydrates.



References

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