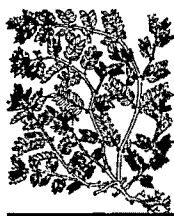




CHAPTER IX

Analysis of Ascarbic Acid and
water content
in *Tribulus terrestris* Linn



.Analysis of Ascarbic acid and water Content in Trhibulus Terrestris Linn

Adsorbic acid is widely distributed in both points and animals. In plants it is mainly found in citrous fruits like lemons, oranges, black carrots etc. It is also found in green leaf, stem and and also in fruits of *T. Terrestris L.*

ISOLATION:

The substance rich in Riboflavin is extracted repeateadly with water. To this solution netural lead acetate is added to precipitats most of the impuri-ties. After filtration, the filtrate is again treated with aa lead salt at pH 7.6 (which is maintained by adding ammonia) to precipitate Ascarbic Acid as as lead salt which is removed by when Ascarbic Acid goes into solution whereas lead is precipitated as $PbSO_4$. After filtration, the filtrate containing Ascarbic Acid is concentrated under vacuum and then treated with organic solvents (acetone, alcohol, ether) to precipitate impurities. Finally, the purified filtrate when sub-jected to distillation, yields pure Ascorbic Acid which is recrytallised from CH_3OH .

CONSTITUTION OF ASCORBIC ACID:

This was elucidated by Haworth, Hirst and co-workers (1933) on the basis of following facts:



1. Molecular formula: From analytical the molecular formula of ascorbic acid has been found to be $C_6H_8O_6$.

2. Presence of Keto-enol system: (i) Ozonolysis of ascorbic acid takes place without producing fragments, indicating that it contains one double bond.

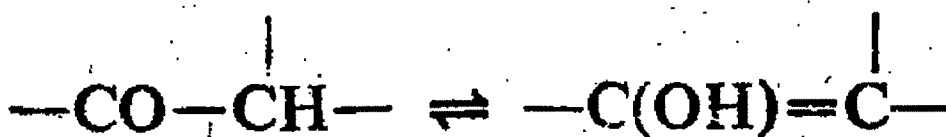
(ii) Ascorbic acid also acts as a strong reducing agent.

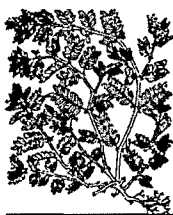
(iii) With ferric chloride solution, ascorbic acid gives violet coloration, indicating the presence of -OH group in it.

(iv) With phenylhydrazine ascorbic acid yields phenyl hydrazone, indicating that it contains $>C=O$ group.

(v) As ascorbic acid does not restore coloration of Schiff's reagent, it does not contain aldehyde group.

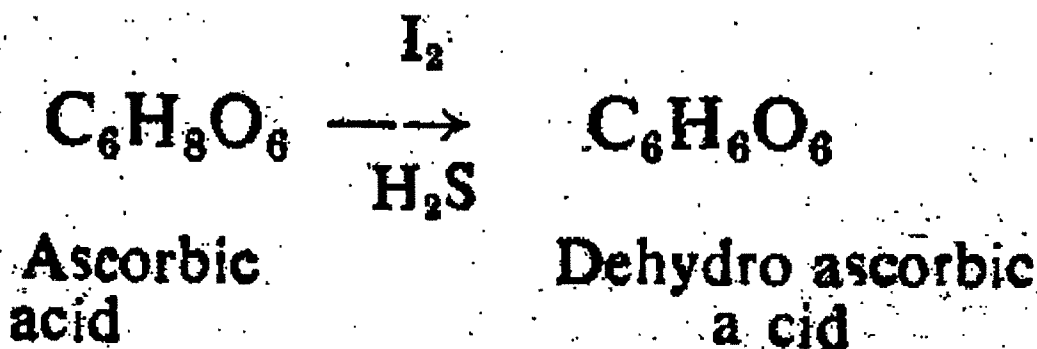
All the above facts [(i) to (iv)] reveal that a keto-enol system is present in ascorbic acid, *i.e.*,





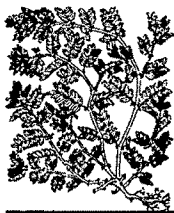
furfuraldehyde. This reaction resembles the conversion of an aldopentose into furfural. Therefore, similar to aldopentose, ascorbic acid must contain at least five carbon atoms in a straight chain, and also that there are a number of hydroxyl groups present (cf. the pentoses) in it.

5. *Presence of hydroxy groups:* Ascorbic acid, when oxidised with iodine in alkaline solution, yields dehydro-ascorbic acid $C_6H_6O_6$. The latter can be reduced back to ascorbic acid by passing HgS gas into it.



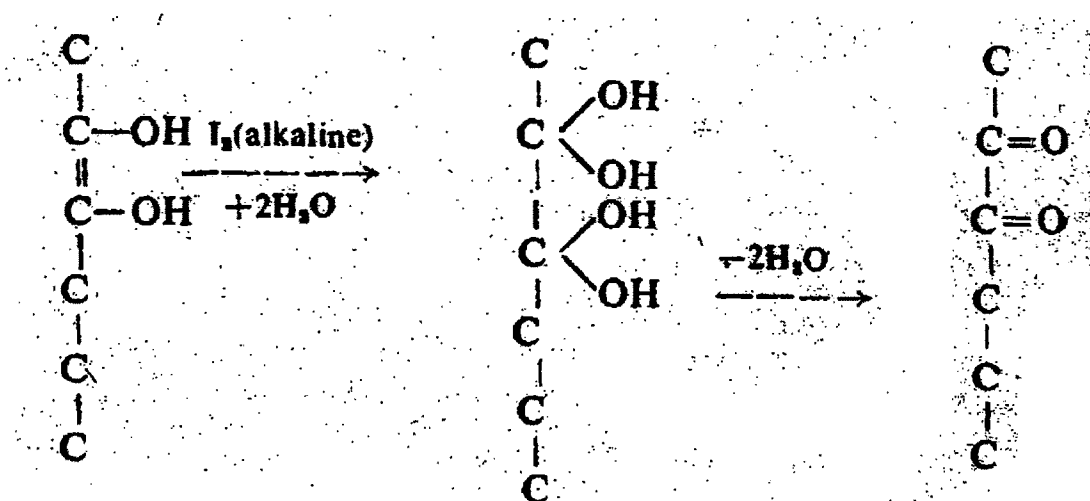
As the oxidation of ascorbic acid involves the elimination of two hydrogen atoms with the formation of the neutral dehydroascorbic acid, this indicates the presence of two enolic hydroxyl groups.

The above is supported by the fact that ascorbic acid when treated with diazomethane yields a dimethyl derivative, indicating the presence of two enolic hydroxyl groups (Diazomethane is a specific reagent for the methylation of enolic groups). Moreover, ascorbic acid yields a violet colour with $FeCl_3$

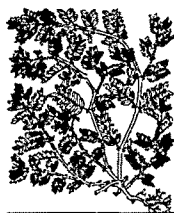


which is a characteristic of enolic groups. Thus, if *dearly* indicates that there are two enolic hydroxyl groups in ascorbic acid.

Dehydroascorbic acid does not give enolic reactions but forms an osazone with phenylhydrazine indicating that two carbonyl groups are present adjacent to each other. Thus, the reversible-oxidation may be explained by writing the part structure of ascorbic acid as (I).



When the dimethyl derivative of ascorbic acid (obtained 'by treating it with diazomethane) is further methylated with methyl iodide in the presence of dry silver oxide, two further methyl groups are added and since all four methyl groups behave as methyl ethers, it therefore follows that two alcoholic groups are present in dimethyl ascorbic acid. Presence of two more alcoholic groups is also confirmed by the fact that the dimethyl derivative yields further a



di. derivative with p-nitrobenzoyl chloride.

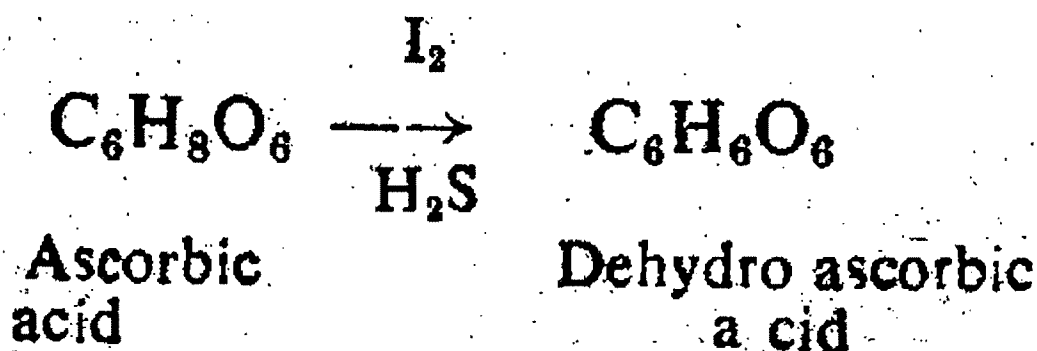
The methyl ether of ascorbic acid when oxidised with lead tetra-acetate gives formaldehyde as one of the products of oxidation. The formation of formaldehyde indicates that dimethylascorbic acid contains two hydroxyl groups, one of which is primary alcoholic in nature. Ascorbic acid as well as its dimethyl-derivative condense—with acetone to yield monoisopropylidene derivative indicating again that ascorbic acid contain two vicinal alcoholic hydroxyl groups and further one of them is a primary alcoholic group,

Moreover, the presence of total four hydroxy groups in ascorbic acid has been proved by the formation of tetra-acetate and tetramethyl derivatives on acetylation and methylation ($\text{Ag}_2\text{O}/\text{CH}_3\text{I}$) respectively.

Further the dimethyl derivative obtained by treatment of ascorbic acid with diazomethane did not give CH_3OH on treatment with alkali. This confirms the absence of a free $-\text{COOH}$ group in ascorbic acid [*c.f.* see (3) above].



6. *Presence of lactone ring.* It is already stated that ascorbic acid on oxidation with aqueous iodine solution yields dehydroascorbic acid which on mHd reduction by H_2S gives back ascorbic acid-

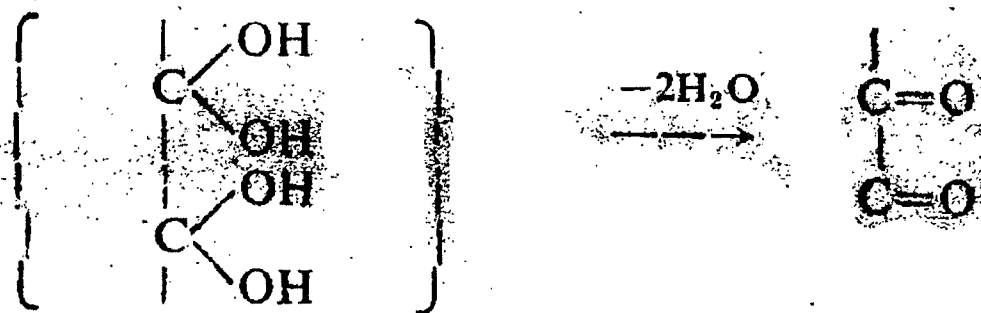


During the oxidation of ascorbic acid, there occurs the removal of two hydrogen atoms, resulting in the formation of dehydroascorbic acid which is neutral and behaves as the lactone of a monobasic hydroxy acid. Also dehydroascorbic acid on reduction with H_2S is reconverted into ascorbic acid.

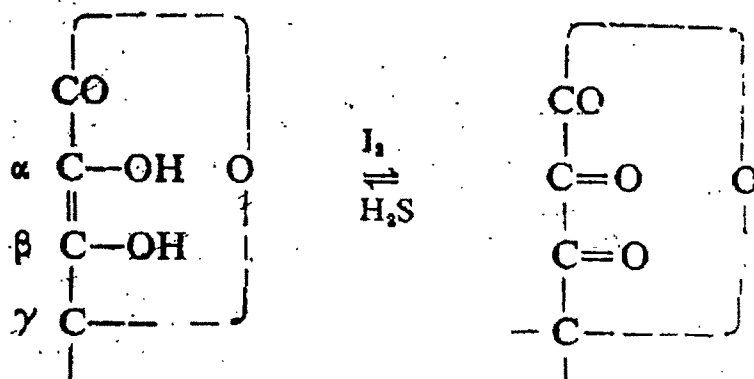
As the above oxidation-reduction process is carried out with mild reagents, it leads to the conclusion that ascorbic acid like its oxidation product dehydroascorbic acid (which is a lactone) is a lactone and not an acid. Now the question arises : *How does this explain the salt-forming property of ascorbic acid?* One possible explanation for this is that the salt-forming property is due to the presence of an enol group, the presence of which has



already been indicated. As all the preceding reactions are characteristics of hydroxy ketones, it means that ascorbic acid must contain an α -hydroxy ketone grouping.

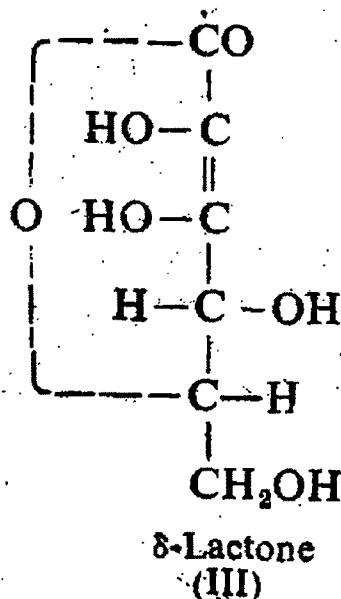
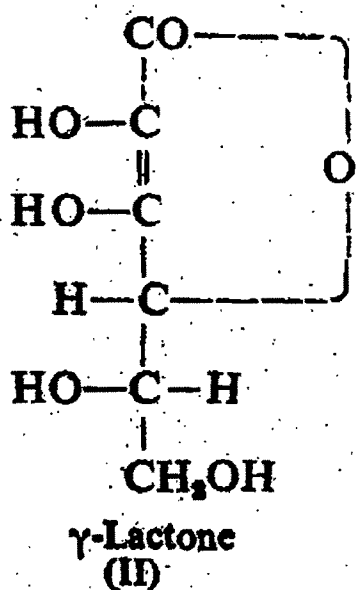


On the basis of the above facts, the reversible oxidation of ascorbic acid may be written as follows ;





On the basis of above facts the structure of the ascorbic acid may be either of the following two structures:



On the basis of structure (II), the foregoing reactions may be explained as follows :

