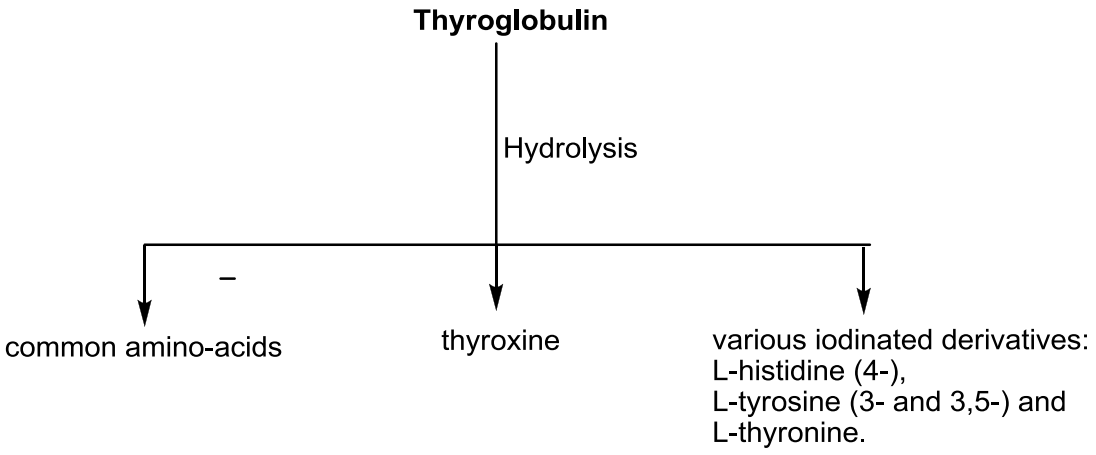
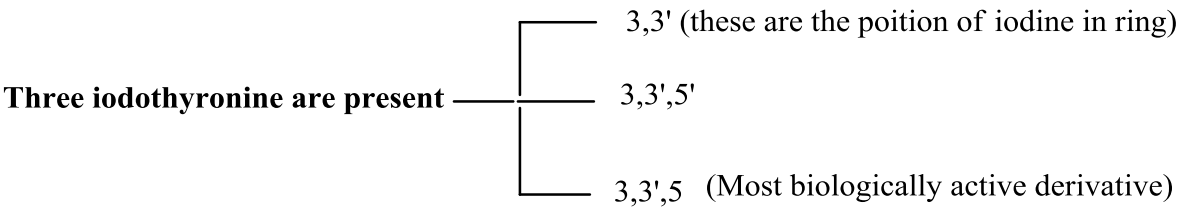
Thyroxine

- It is a hormone consist of an iodine based protein called *thyroglobulin:* occurs in the thyroid gland.
- First isolated by **Kandall (1919)** and later by Harington (1930) as a white crystalline solid, melting point 235^{o}C.
- On hydrolysis, thyroglobulin yields the followings.





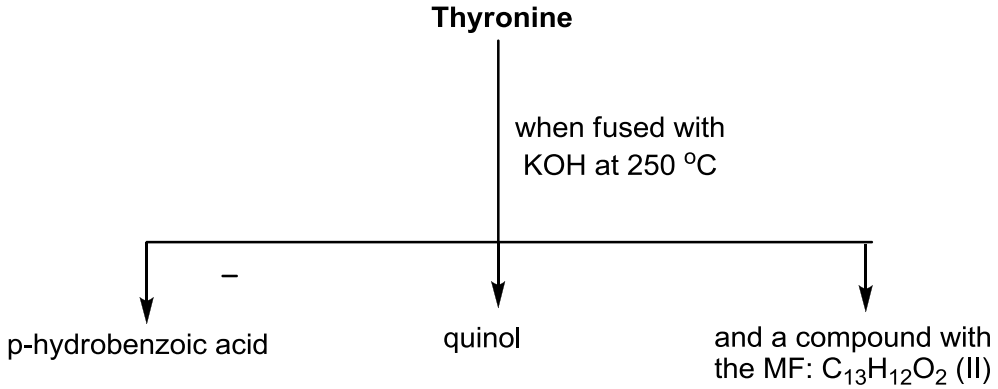
Structure Determination

Thyroxine: structure was established by Harington (1926).

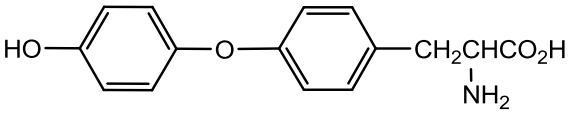
The molecular formula: $C_{15}H_{11}I_4NO_4$

- Thyroxine form **thyronine** (thyronin: $C_{15}H_{15}NO_4$) when treated in alkaline solution with hydrogen in the presence of colloidal palladium, wherein *iodine is replaced by hydrogen*.
- Thyronine behaves as a *phenol* and *an amino-acid*.
- On fusion with potassium hydroxide in an atmosphere of hydrogen, thyronine gives a mixture of *p-hydroxybenzoic acid, quinol, oxalic acid* and *ammonia*.

- When fused with potassium hydroxide at 250^{o}C



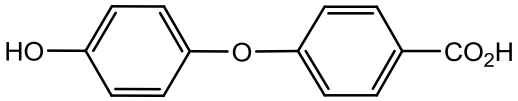
Therefore, a structure of thyronine which would give all these products is (I).



(I)

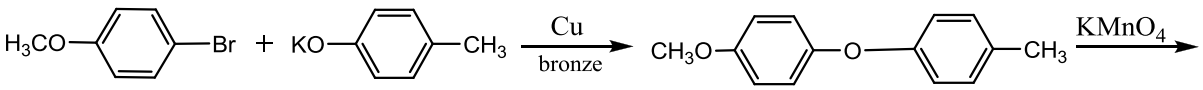
thyronine

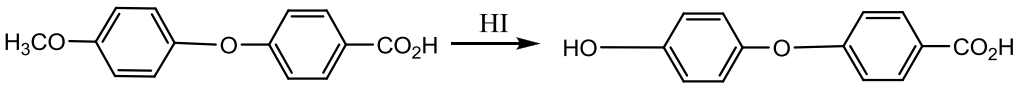
When thyronine (assumed structure (I)) was subjected to the Hofmann exhaustive methylation followed by oxidation, the final product would be (III).



(III)

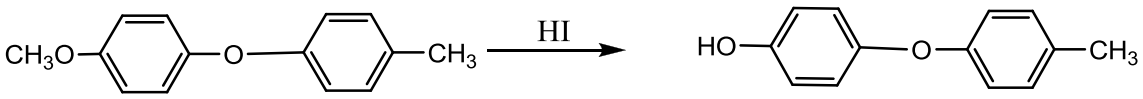
The structure of (III) was further confirmed by synthesis, starting from <u>*p*-bromoanisole and *p*-cresol</u> as below.





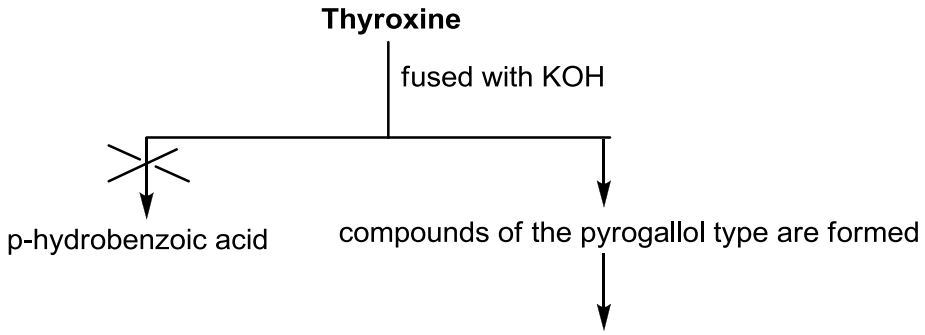
(III)

- When 4-methoxy-4’-methyldiphenyl ether is heated with hydriodic acid, compound (II) is obtained; thus the structure of (II) is also established.

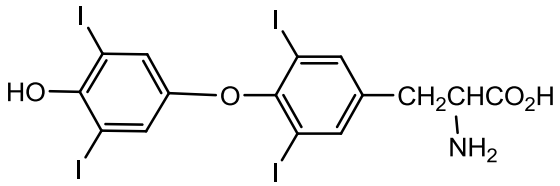


(II)

- When thyroxine is fussed with potassium hydroxide



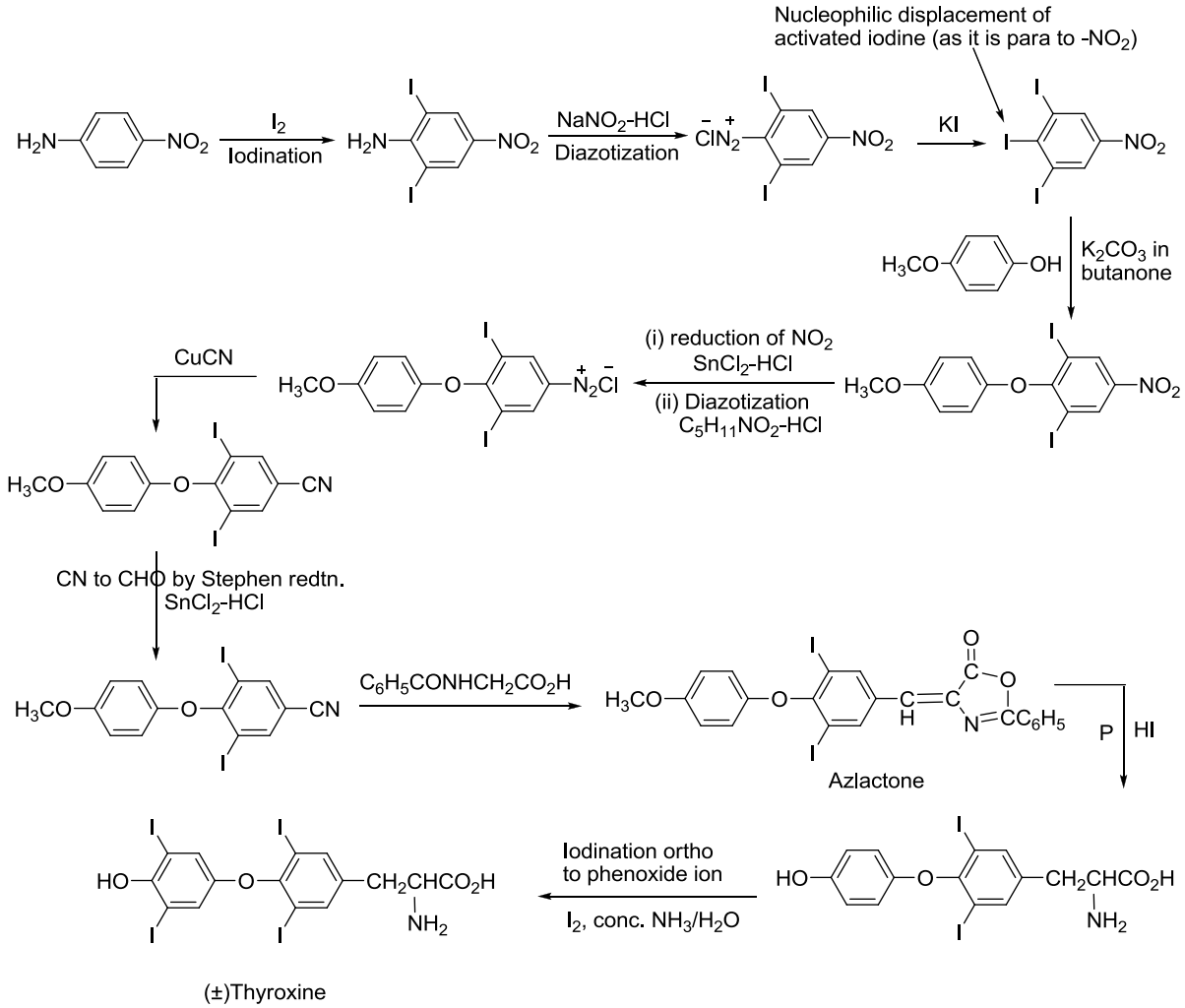
These facts suggest that two atoms of iodine are adjacent to the hydroxyl group, and that two remaining iodine atoms are in the other benzene ring. This, together with the analogy with di-iodotyrosine, leads to the suggestion that thyroxine is (IV).



(IV)

thyroxine

Synthesis of thyroxine: Harington *et al*., in 1927 confirmed the structure of thyroxine by synthesis from 4-nitro aniline.



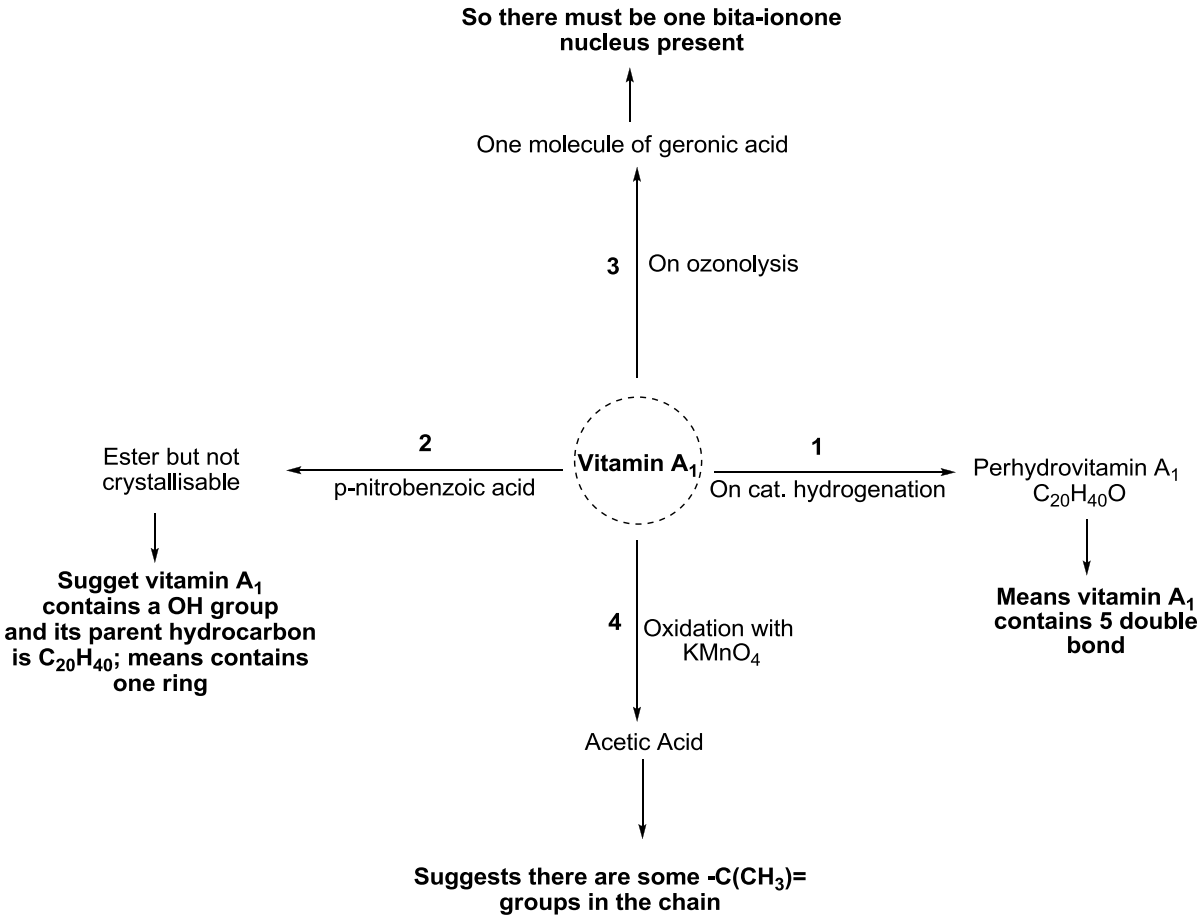
After racemic modification resolution *via* the formyl derivative, it was shown that this amino-acid belonged to the L-series.

Vitamin A or Retinol or Axerophthol

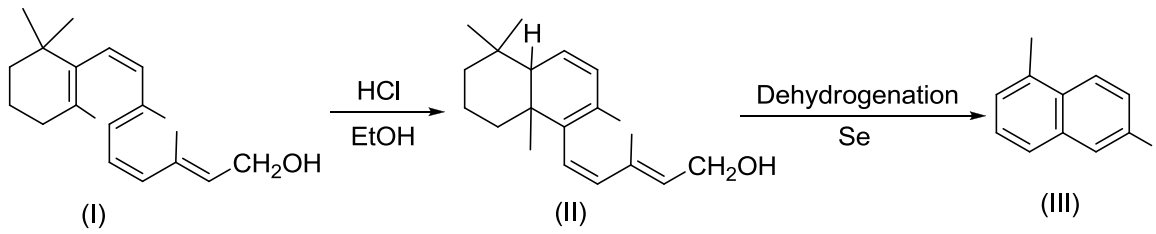
- **Molecular formula:** $C_{20}H_{30}O$
- Vitamin A usually referred to as vitamin A_1 since a second compound, known as vitamin A_2, has been isolated.
- These vitamins are **diterpenoids** and belonging to the **apocarotenoid group**.
- It is known to influence growth in animals and increases resistance to disease.
- It's deficiency causes *night blindness* in human diet, and a prolonged deficiency leads to *xerophthalmia*.
- Vitamin A_1 occurs free and as esters in fats, in fish livers and in blood. It was originally isolated as a viscous yellow oil, but later it was obtained as a crystalline solid with m.p. 63-64^oC.
- it is estimated by the blue colour reaction with antimony trichloride solution in in chloroform (Carr-Price reaction).
- The IUPAC name of vitamin A (A_1) is **retinol**; that of the corresponding aldehydes retinal and that of the corresponding acid is retinoic acid.
- Carotenoids change into vitamin A_l in the intestinal mucosa, and the potency of α- and γ-carotenes is half that of β-carotene. This provitamin nature of β-carotene led to the suggestion that vitamin A_l is half the molecule of β-carotene.

Structure Determination

- When following reactions were performed on vitamin A_1, these suggested some facts.



- All of the above facts led to the suggestion that vitamin A_1 is half the β-carotene structure. When heated with an ethanolic solution of hydrogen chloride, vitamin A_1 is converted into some compound (II) which, on dehydrogenation with selenium forms 1,6-dimethyl-naphthalene (III). Heilbron assumed (I) as the structure of vitamin A_1, and explained the course of the reaction as follows:

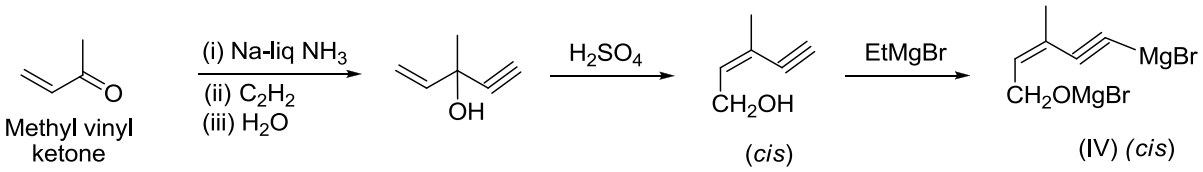


Synthesized perhydrovitamin A_1 from β-ionone was found to be identical with the compound obtained by reducing vitamin A_1, which support the structure assigned to vitamin A_1. Finally the structure of vitamin A_1 was confirmed by its synthesis done by several groups.

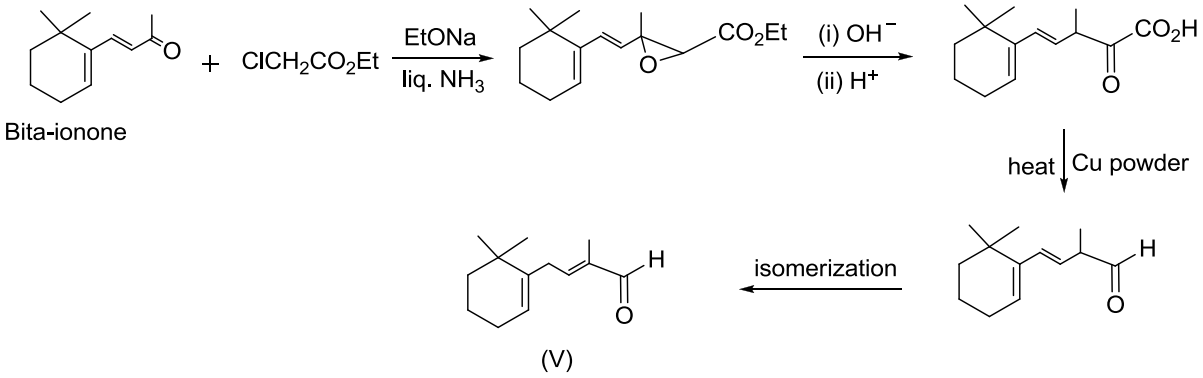
Synthesis of Vitamin A_1:

1. **By Isler *et al.* (1947):** This group started with methyl vinyl ketone to produce compound (IV) and then reacted with (V).

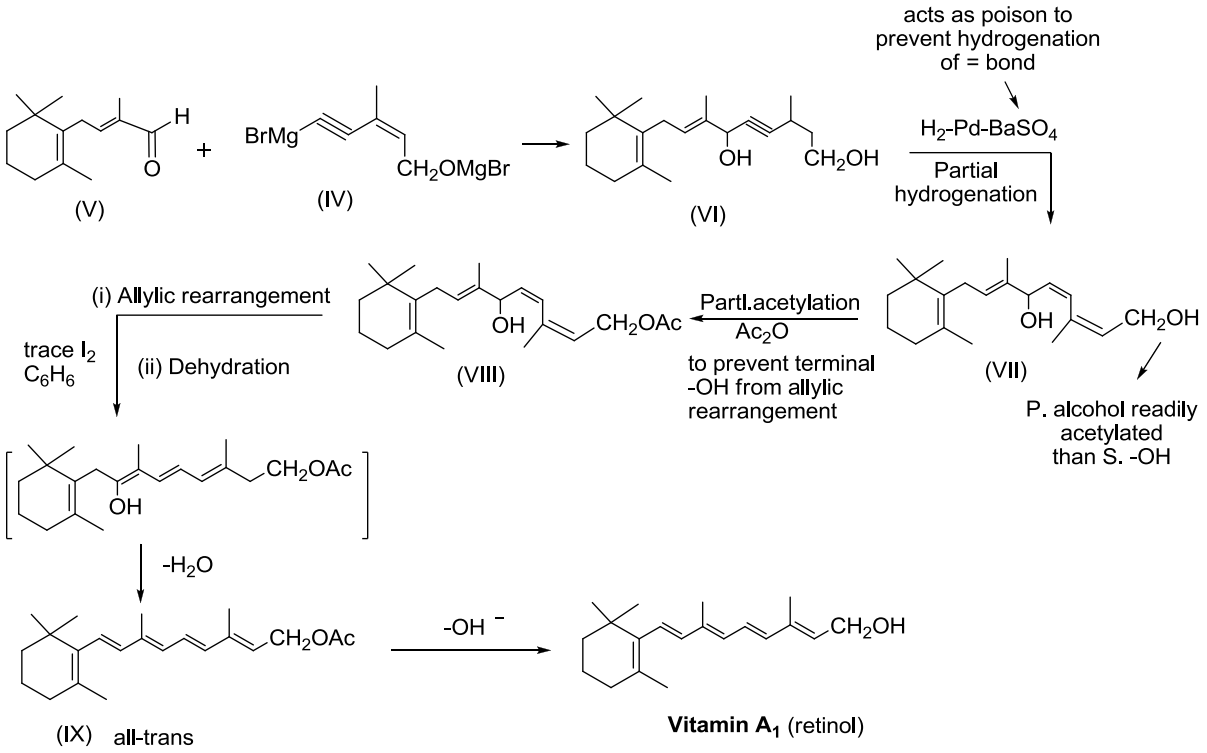
(a) Preparation of (IV)



(b) Preparation of (V)



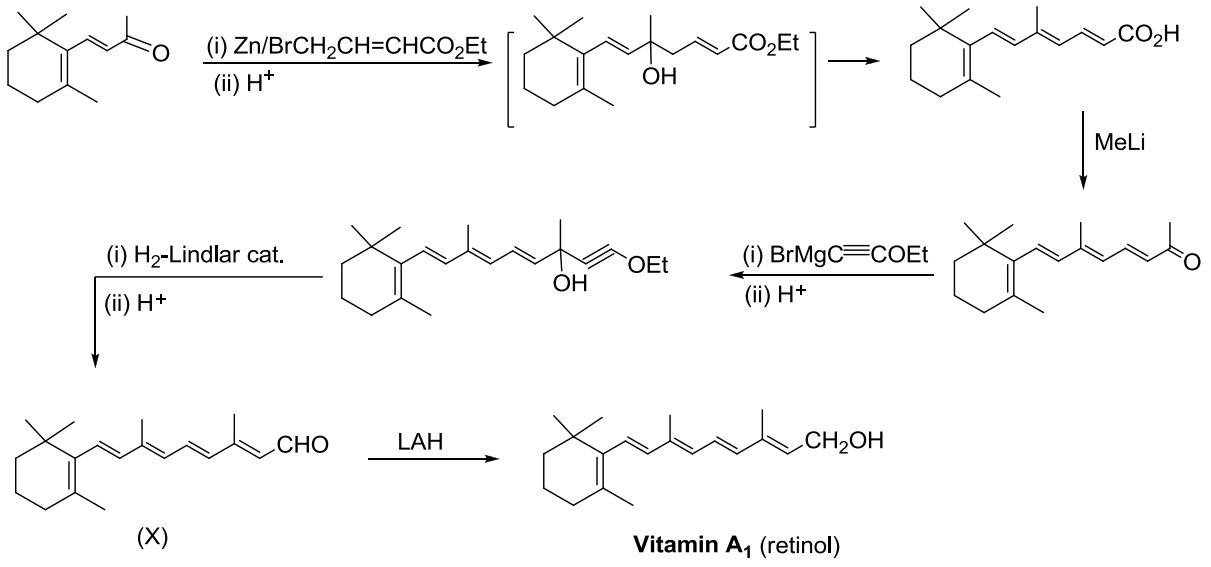
Combining (IV) and (V)



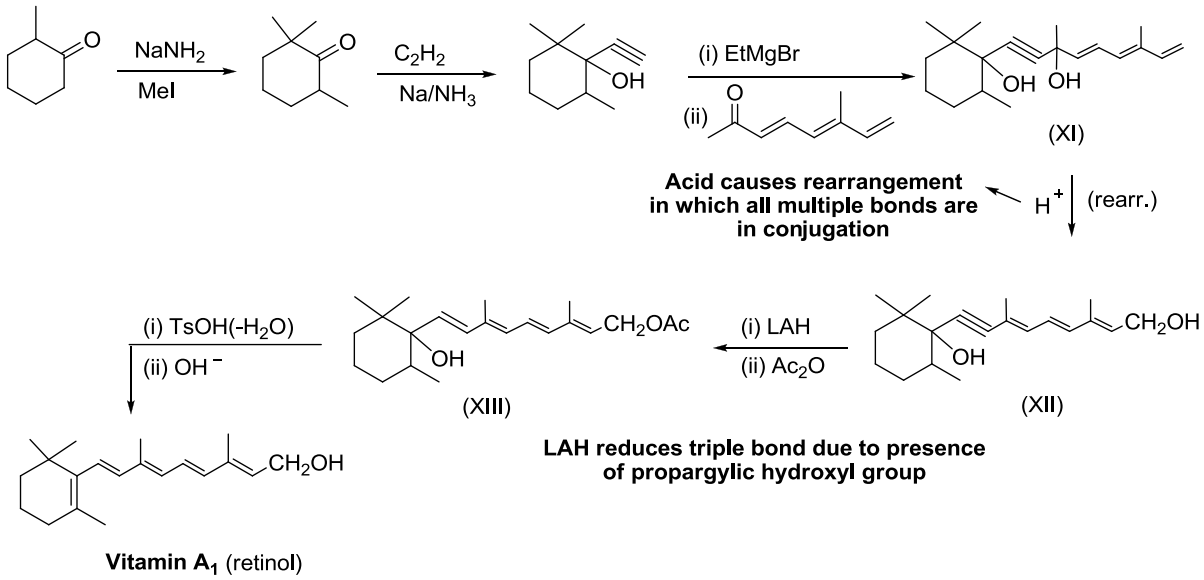
- The crude vitamin A_1 was purified *via* its ester with anthraquinone-2-carboxylic acid, and was shown to be identical with natural vitamin A_1.

- Lindlar catalyst (Pd-$CaCO_3$ + lead acetate for partially inactivation + quinoline) is used for partial hydrogenation of triple bonds with better result in terms of yield.

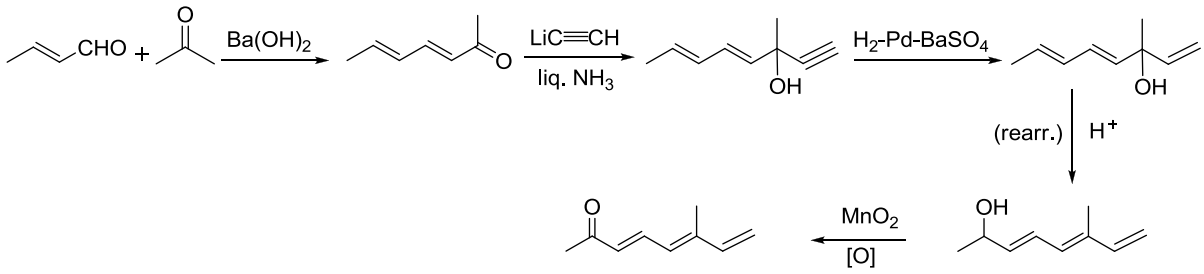
2. **By van Dorp *et al*. (1949):** They prepared retinal$_1$ (X), which was then reduced by mean of lithium aluminium hydride to vitamin A_1; β-ionone and ethyl γ-bromocrotonate were the starting materials.



3. **By Attenburrow *et al*. (1952):** They also synthesised vitamin A_1 starting from 2-methylcyclohexanone.

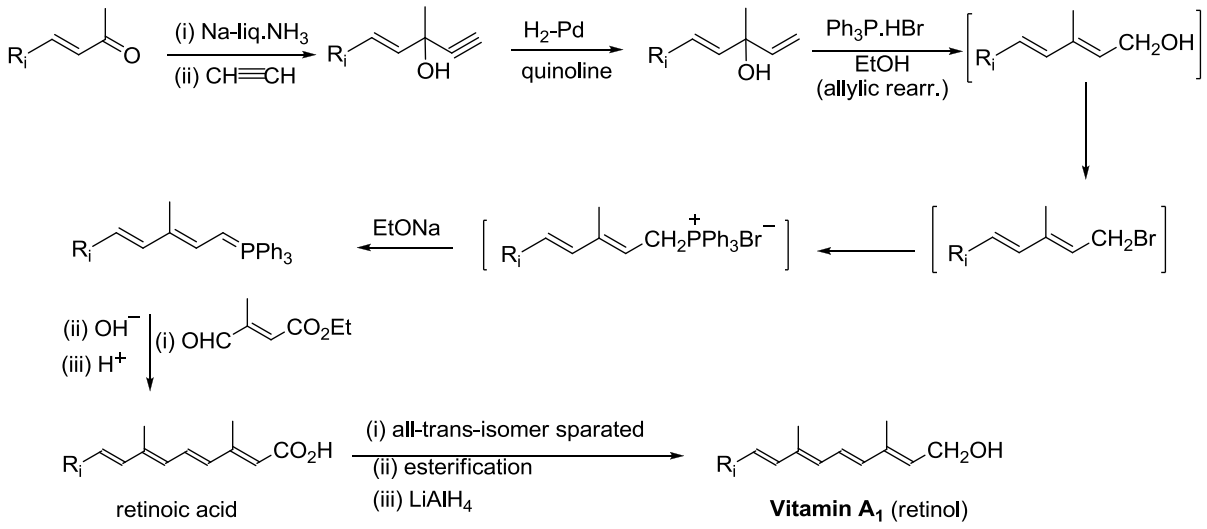


The unsaturated ketone used in the third stage in above synthesis was prepared as follows:

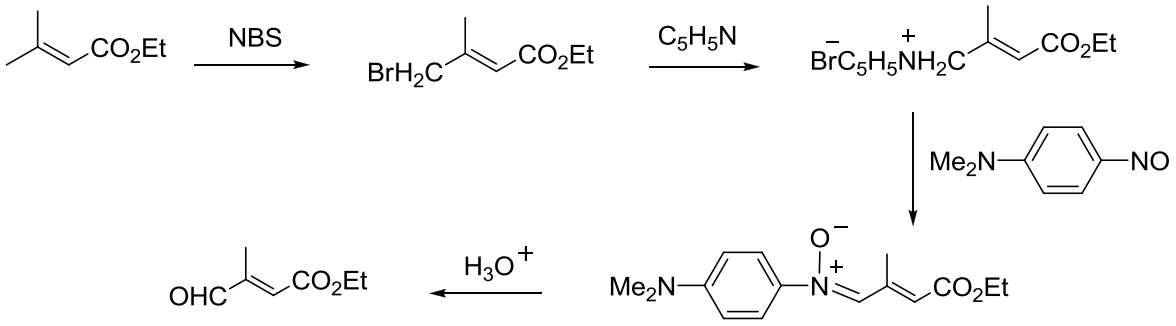


4. **By Pommer *et al*. (1958,1959):** They have synthesised vitamin A_1 *via* a Wittig reaction.

$R_{i=}$ β-ionone ring =  .



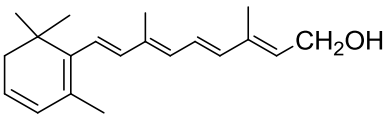
The are several methods reported for synthesis of ethyl γ-oxocrotonate used in the above synthesis. One method developed by Sisido *et al*. (1960) uses *N*-bromosuccinimide (NBS) and the synthesis is an example of the Krohnke aldehyde synthesis (1936-1939).



- **Synthetic vitamin A_1 is now a commercial produce by Isler method.**

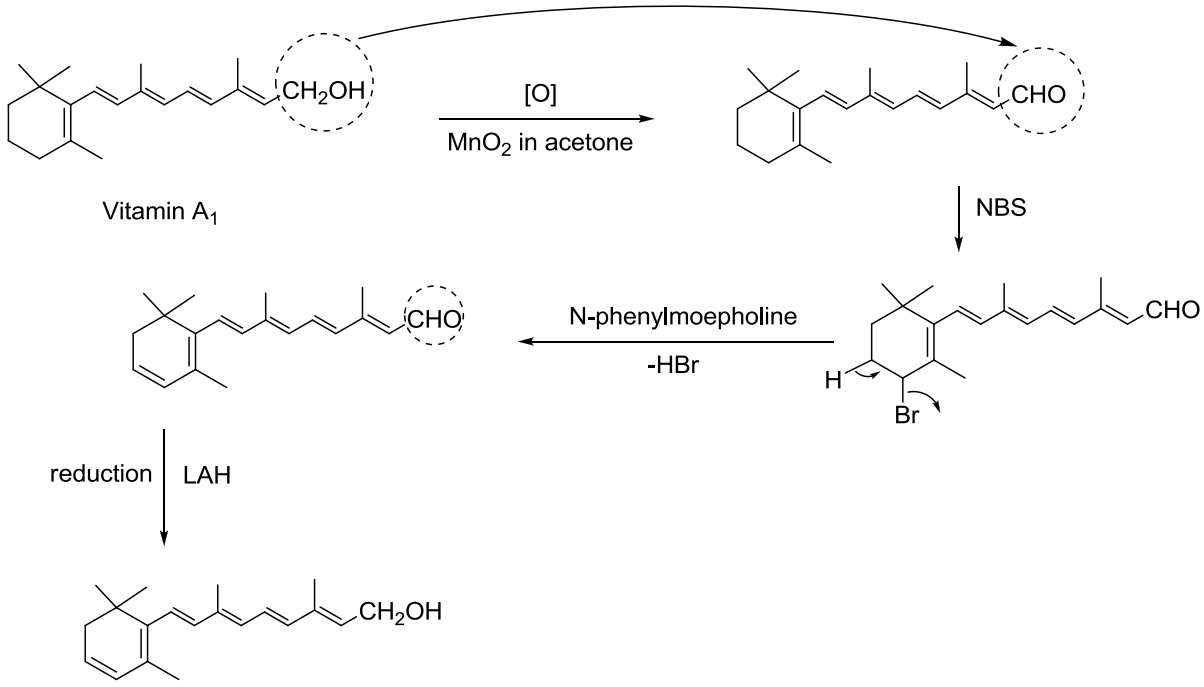
Vitamin A_2

It is a second vitamin A and was isolated from natural sources, and synthesised by Jones *et al*.(1951,1952); it is dehydrovitamin A_1 (3,4-dehydroretinol).



Vitamin A_2

Synthesis of vitamin A_2 by Jones *et al.*(1955): They prepared vitamin A_2 from vitamin A_1 as follows:



Vitamin A_2

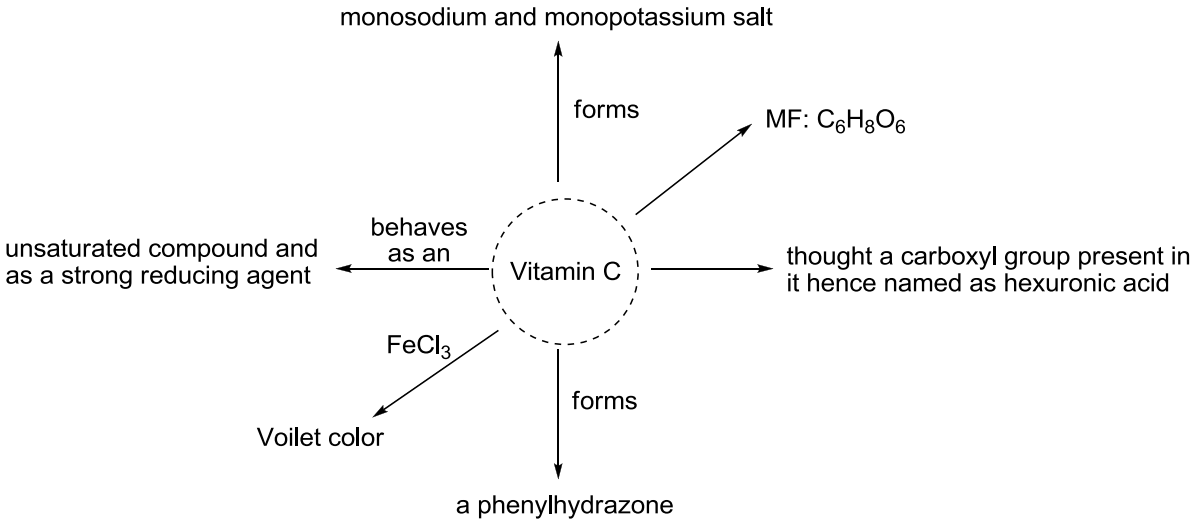
- Vitamin A_2, m.p. 63-65^{o}C

Vitamin C or L (+)-Ascorbic Acid

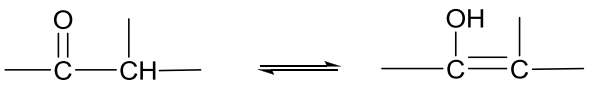
- Vitamin C closely related to the monosaccharides.
- Its deficiency in diet **scurvy**.
- Hawkins (1593) discovered that oranges and lemon were effective for treating scurvy, a disease particularly prevalent among seamen.
- Holst and Frolich (1907) produced experimental scurvy in gunia-pigs.
- Then Szent-Gyorgi (1928) extracted a crystalline substance from various sources, and found that it had antiscorbutic properties. This compound originally called *hexuronic acid*, and later was shown to be identical with vitamin C, m.p. 192^{o}C, of +24^{o}.

Structure Elucidation

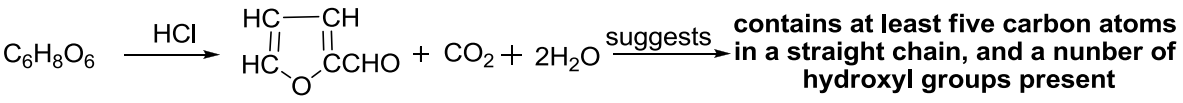
Haworth, Hirstand their co-workers (1932,1933): Elucidated the structure of vitamin C.

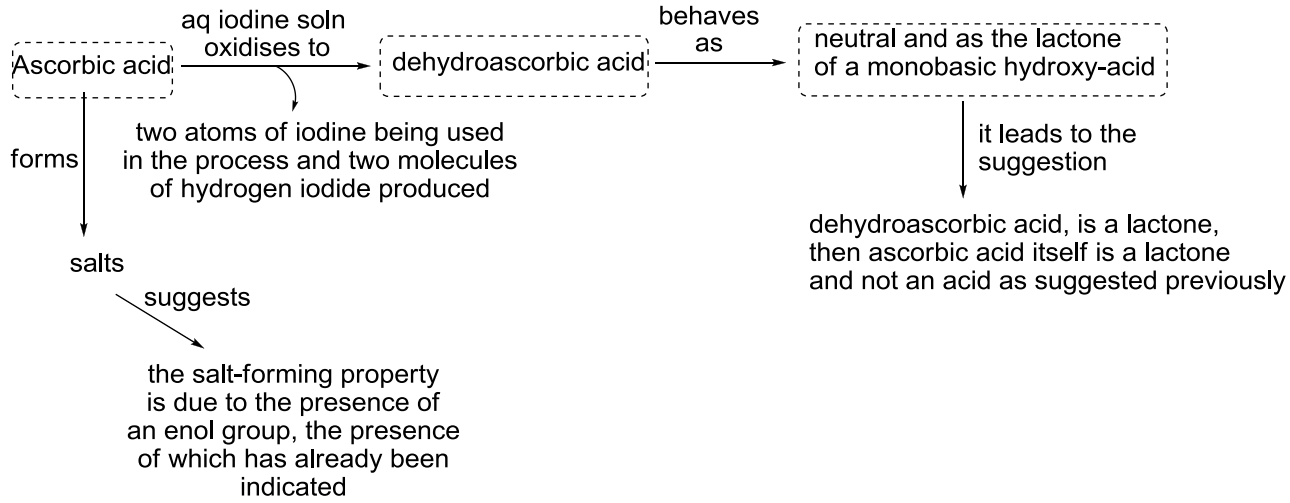


All these suggest that a keto-enol system is present, *i.e.*,

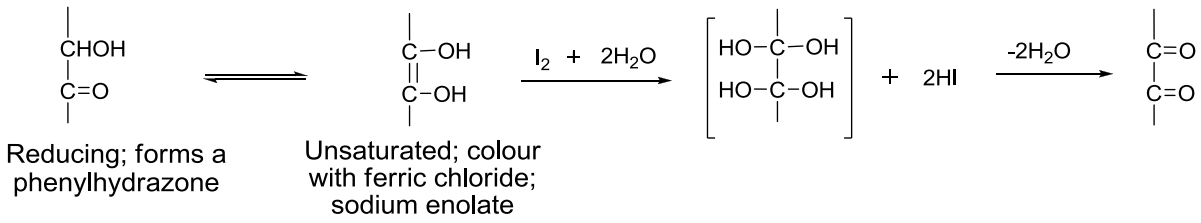


Now, when boiled with hydrochloric acid, ascorbic acid gives a quantitative yield of furfuraldehyde:

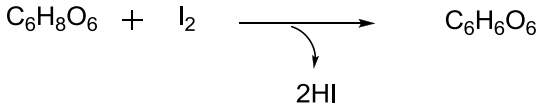




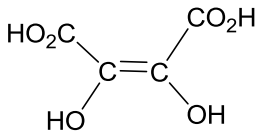
Thus all the above reactions can be explained by the presence of an hydroxyketone grouping in ascorbic acid:



The final result is the removal of two hydrogen atoms to form dehydroascorbic acid.

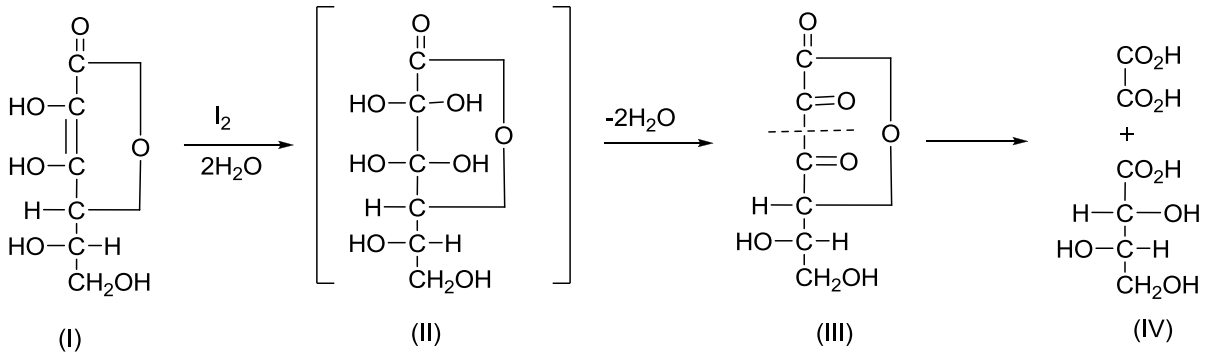


They are known to occur with dihydroxymaleic acid; hence by analogy with this compound, the explanation offered for the reactions of ascorbic acid is very strongly supported.



dihydroxymaleic acid

Oxalic and L-threonic acid (IV) was identified by methylation and then conversion into the crystalline amide after their quantitative production from dehydroascorbic acid by oxidation with sodium hypoiodite. L-Threonic acid was found to be identical with tri-*O*-methyl-L-threonamide (obtained from L-threose). Further evidence about nature of product (IV) is that it gives D(+)-tartaric acid on oxidation with nitric acid. The oxalic and L-threonic acids formation suggests that dehydroascorbic acid is (III), lactone of 2,3-diketo-L-gulonic acid. Hence, it can be assumed that (I) is the structure of ascorbic acid, the previous reactions as follows, dehydroascorbic acid being formed *via* (II).

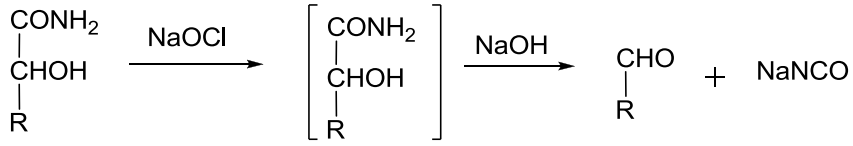


(I) (II) (III) (IV)

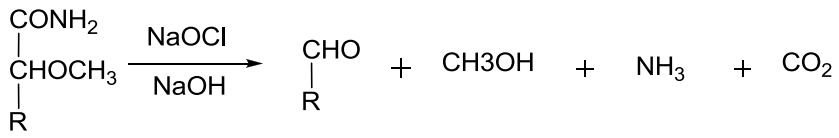
It was assumed to be five-and not six-membered ring in ascorbic acid as lactone (*e.i.*,ascorbic acid) is stable in alkali. However, same final products would also have been obtained had the ring been six-membered. Stucture (I), however, has been amply confirmed by other analytical evidence. Ascorbic acid get converted into dimethylascorbic acid (V) by diazomethane; since diazomethane readily methylates acidic (in this case, enolic) hydroxyl groups these two methoxyl groups are most likely on C-2 and C-3. This dimethyl derivative is neutral, and form a sodium salt without the elimination of a methyl group after dissolution in aqueous NaOH; so cannot be a carbomethoxyl group present, and most likely two enolic hydroxyl group are present. Opening of a lactone ring is due to the formation of the more sodium salt from the neutral compound. With methyl iodide in the presence of dry silver oxide, this dimethyl derivative is methylated, two further methyl groups are introduce to give (VI), therefore two alcoholic groups are present in dimethylascorbic acid because all four methyl

groups behave as methyl ethers. O*ne* neutral substance containing the *same* number of carbon atoms because its precursor is produced on ozonolysis of this tetramethyl compound. Since ozonolysis of a carbon-carbon double bond result is scission of that bond, there must be a ring system present in the tetramethyl compound to hold together the two fragments (VII). Oxalic acid and dimethyl-$_L$-threonic acid (VIII) is obtained from this ozonised product, on hydrolysis with barium hydroxide. These products in all contains three carboxyl groups, and as ozonolysis of a double bond produces only two, the third carboxyl group must engaged *as a lactone* so that ascorbic acid should behave as a neutral compound.

The size of the ring in ascorbic acid is the structure of this dimethyl-$_L$-threonic acid, the nature of which has been ascertained as follows. On methylation, followed by conversation to the amide, trimethyl-$_L$-threonamide. Thus this dimethyl compound, which was unknown when isolated, is a dimethyl-$_L$-threonic acid; but where are the two methoxy groups? Their positions were ascertained by means of the Weerman test which is used for showing the presence of a *free* hydroxyl group in the α-position to an amide group, *i.e.*, in an α-hydroxy-amide. Treatment of a hydroxy-amide with alkaline sodium hypochlorite gives an aldehyde and *sodium cyanate* if there is a *free* hydroxyl group on the α-carbon atom.

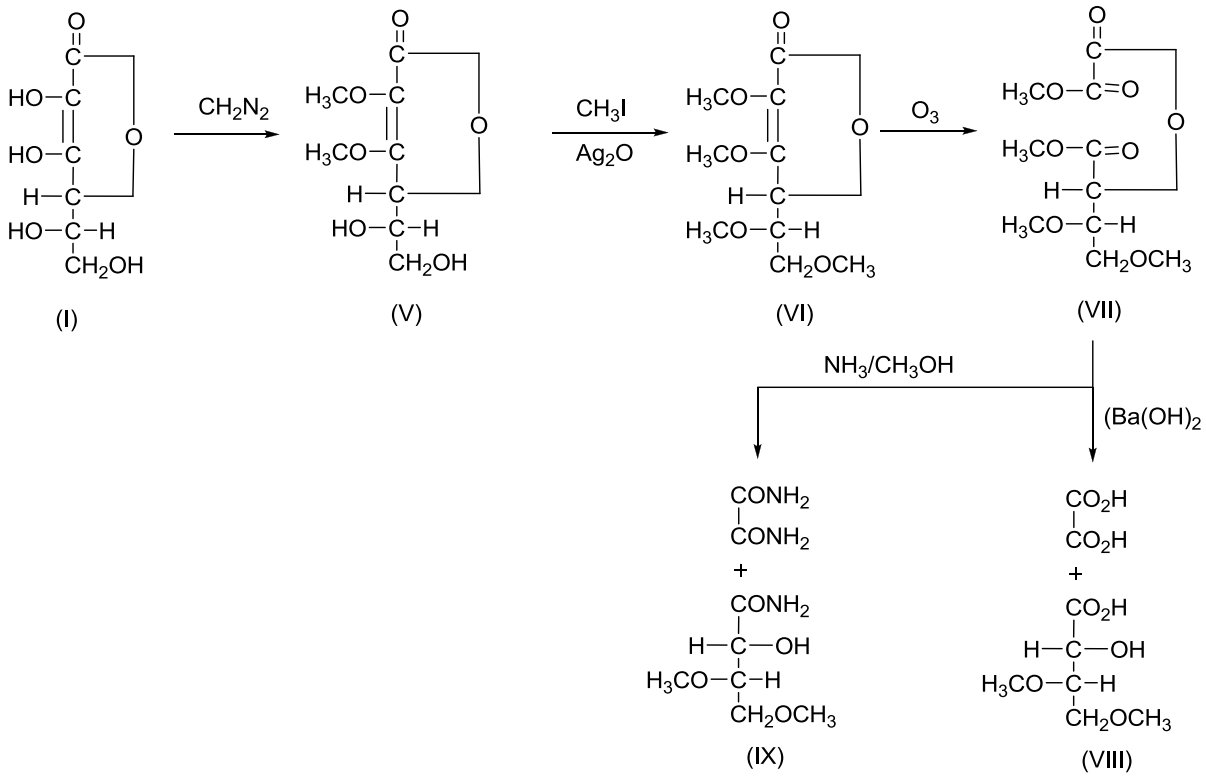


If free hydroxyl group are absent on the α-carbon atom, then aldehyde, methanol, ammonia and carbon dioxide are produced on treatment with alkaline sodium hypochlorite.

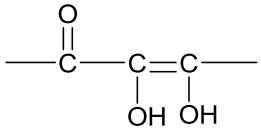


The dimethylthreonic acid derived from the ozonised product was converted into the amide (XI), and gave sodium cyanate as one of the products on Weerman test. This suggests a free α-hydroxyl group, and consequently must be 3,4-di-*O*-methyl-$_L$-threonic acid (VIII) in this dimethylthreonic acid. It implies that the lactone ring in ascorbic acid must be γ-, because δ-lactone could not have given (VIII). Both amide (IX) and oxamide obtained together with, by

the action of ammonia in methanol on the ozonised product (VII). All the foregoing facts can be shown as below:

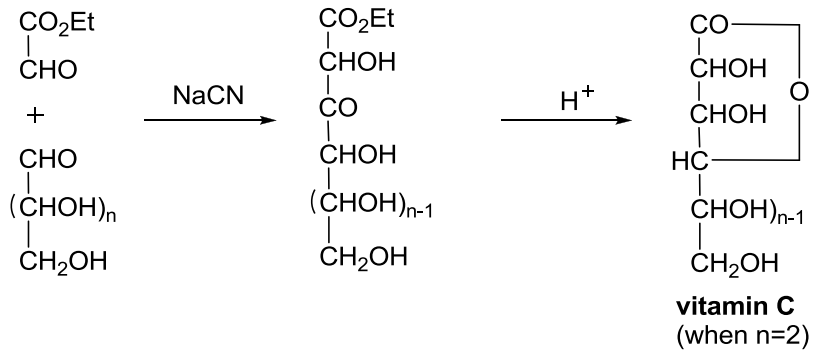


Ascorbic acid does not contain a 'normal' carbonyl group as it is *not* reduced by lithium aluminium hydride. It has been found that lithium aluminium hydride does not reduce all **reductones**. Reductones are compound which contain the ene-α-diol-α-carbonyl group like following.



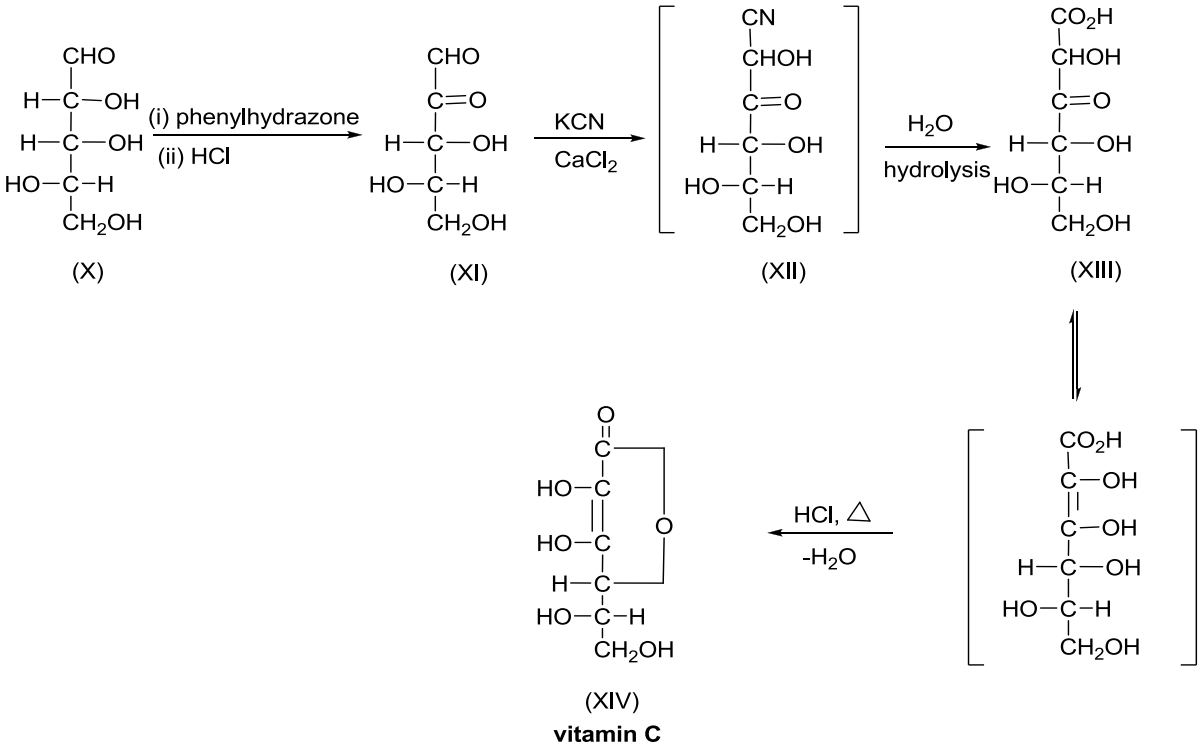
Synthesis of ascorbic acid

a) Several methods are reported for synthesis of ascorbic acid. A common method used for the preparation of ascorbic acid rely on the condensation between polyhydroxy-aldehyde and ethyl glyoxylate in the presence of sodium cyanide (benzoin-type condensation). The intermediate 3-oxo-derivative which was not isolated then hydrolysed with acid to give the product.



When L-threose (n = 2) used, vitamin C is obtained.

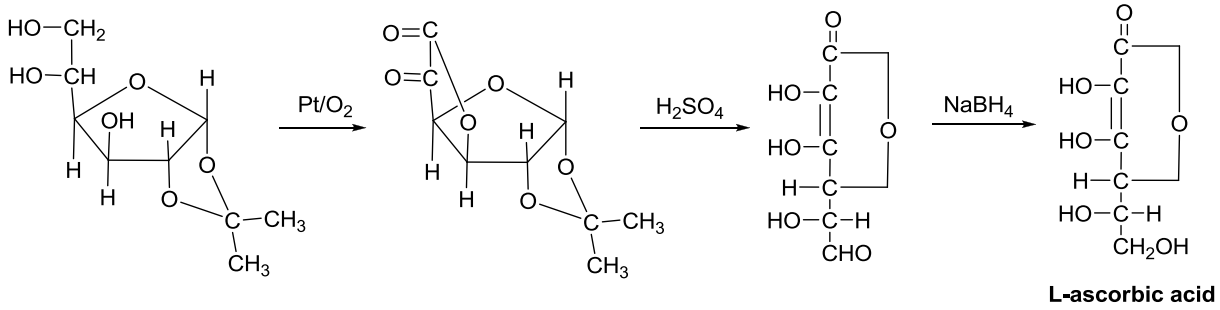
b) Haworth and Hirst (1933), converted L-Lyxose, (X) into L(-)-xylosone (XI) by treating with phenylhydrazine followed by hydrolysis of osazone with hydrochloric acid.



Compound (XI) gave the keto-cyanide (XII) on treatment with aqueous potassium cyanided containing calcium chloride in an atmosphere of nitrogen, which hydrolysed spontaneously into *pseudo*-$_{L}$-ascorbic acid (XIII). This gave a quantitative yield of $_{L}$(+)-ascorbic acid (XIV) on hearing for 26 hours with 8 % of hydrochloric acid at 45-50^{o}C.

$_{L}$-Lyxose in the above synthesis was prepared by stepping down $_{D}$-galactose. Reichstein *et al.* (1932) also synthesised $_{L}$-ascorbic acid independently of Haworth and Hirst. In this method $_{L}$-xylose, which was prepared from $_{D}$-glucose, was converted into $_{L}$-xylosone, etc.

c) A much shorter synthesis of $_{L}$-ascorbic acid, from $_{D}$-glucose was introduced by Bakke *et al.* (1971). The oxidation of 1,2-*O*-isopropylidene-$_{D}$-glucofuranose by platinum-oxygen in acid solution, and then product was treated with dilute sulphuric acid followed by reduction with sodium borohydride at *p*H 7 to yield the product.



L-ascorbic acid

Vitamin C is often referred to as $_{L}$-xyloascorbic acid because it is derived from $_{L}$-xylohexulosonic acid (2-ketogulonic acid). Natural vitamin C has more antiscorbutic property than many ascorbic acids synthesised.

d) There are several methods for commercial synthesis of ascorbic acid. In one of them $_{D}$-glucose was converted into to (+)-sorbitol by catalytically hydrogenation which is then subjected to microbiological oxidation (using *Acetobacter suboxydans* or *Acetobacter xylinum*) to convert into (-)-sorbose. Direct oxidation of (-)-sorbose to 2-keto-(-)-gulonic acid with nitric acid can be carried out, but the yield is less than the above. The yield of gulonic acid's is higher by protecting these by means of 2,3-4,6-di-isopropylidene formation because nitric acid oxidises other alcohol groups besides the first. Quantitative oxidation of di-isopropylidene derivative to di-isopropylidene-2-keto-($^{-}$)-gulonic acid by oxygen in the presence of a Pt-C catalyst was found by Gorlich (1955). The next step involves the dissolution of gulonic acid is in mixed solvents (of which chloroform is the main

constituent) and hydrogen chloride passed in. Finally the product, L-ascorbic acid, was purified by charcoaling.