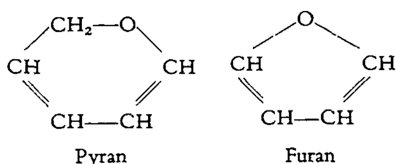
WALTER N. HAWORTH

The structure of carbohydrates and of vitamin C

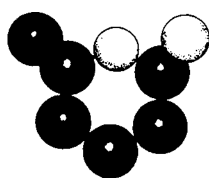
Nobel Lecture, December 11, 1937

The structure of carbohydrates ad of vitamin C

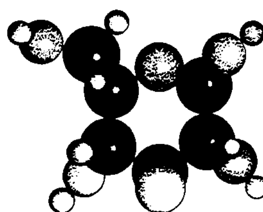
Twenty years ago it could have been said that the wealth of natural products which comprise the carbohydrate group was bewildering in its complexity. Such materials as cellulose, glycogen, and starch seemed almost beyond the range of structural investigation. It was recognized that these products were built up somehow of simple sugars, particularly glucose. But our knowledge of the mode of combination of two or more glucose molecules was doubtful and insecure. This could scarcely have been otherwise inasmuch as it was not until 1925 that a precise structural model of any sugar was clearly and finally determined. The expressions used by Emil Fischer give us the stereochemical relationship of the hexoses and pentoses which he represented as open-chain aldehydes and ketones and these configurational conceptions will always be regarded as classical. It must now be said, however, that sugars of the hexose and pentose series, whether occurring as free isolated substances or assembled as the constituent parts of complex carbohydrates, conform to one of two simple structural models which are related either to pyran or to furan.



The model of glucose which I introduced in 1925 is represented in skeleton form as being built up of a ring of six atoms, five of these being carbon and one oxygen, together with an additional side-chain carbon atom. This I described as the pyranose form. When this model is clothed with its constituent oxygen and hydrogen atoms it then appears as represented in the second picture above where the model of bglucose is portrayed. If we depart from this atomic representation and sketch a formula for α- and β-

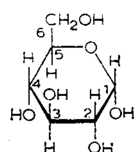


Skeleton model of glucose

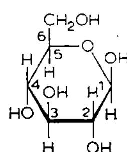


Model of β-glucose

glucose it will be best to have this model in mind and represent it by perspective formulae.

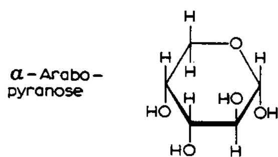


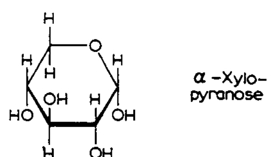
α–Glucopyranose

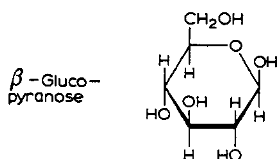


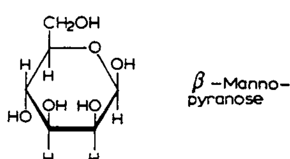
β–Glucopyranose

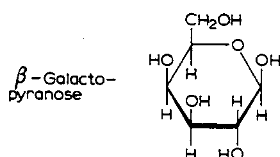
Other normal hexoses have been shown to conform similarly to this same structural plan. Thus the normal varieties of β-mannose, β-galactose, and β-fructose are illustrated below, all of them being pyranose forms, i.e. forms which are based upon the ring structure present in pyran. Similarly the normal pentoses have been shown to be pyranose in structure and their formulations are here given.

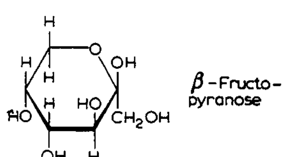




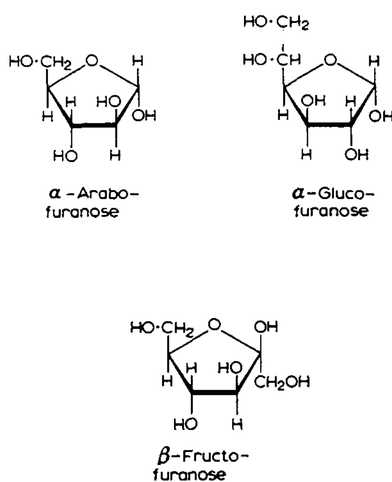




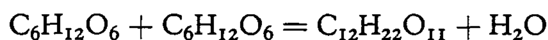




Much less stable forms of pentose and hexose are those which I have described as the furanose forms. Only the derivatives of these have been isolated as homogeneous crystalline substances, e.g. the ethyl- and methyl-glucosides which are hydrolysed with something of the order of 100 times the velocity of the normal methylglucosides. Yet it is in this form that certain sugars occur in a state of combination in Nature, particularly the pentose arabinose and the hexose known as fructose or hevulose. When these sugars are isolated they revert to the normal or pyranose forms which have six-atom rings.



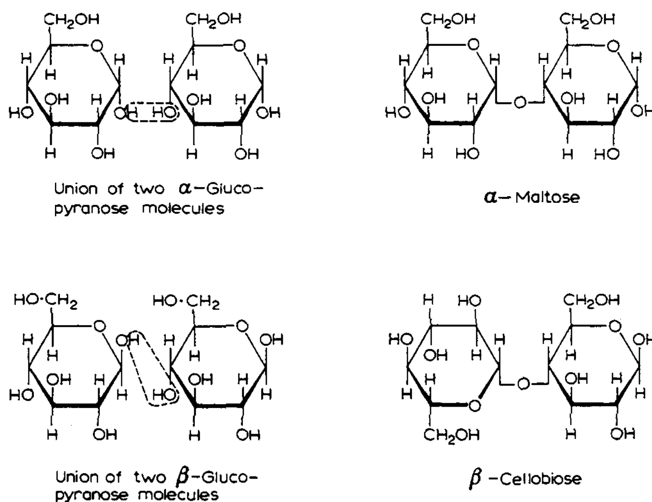
It is clear that these simple sugars acquire wider significance when regarded as the building stones in such complex natural products as cellulose, starch, inulin, or the wood gum known as xylan. Much of my work has been devoted to an inquiry into the manner in which two or more sugars unite with one another or are found united in Nature in the disaccharides such as sucrose, maltose, cellobiose, gentiobiose, melibiose and others. From the picture of simple sugars which I have given it will be evident that there are several ways in which two glucose units may unite, by loss of water, through the intermediary of a common oxygen atom. Investigations conducted during the past 15 years have enabled us to build upon the speculations of Emil Fischer and to arrive at a precise picture for each of the disaccharides. The expression



$$C_6H_{12}O_6 + C_6H_{12}O_6 = C_{12}H_{22}O_{11} + H_2O$$

merely indicates the union of two hexose residues with loss of water to give a biose. Any of the five hydroxyl positions present in a hexose such as glucose are available as a means of attachment to a similar glucose residue. Actually those bioses found in Nature do not exhaust all the possibilities which are available as a means of assembly of pairs of sugar units.

If we utilize a method of numbering the carbon atoms of a pyranose, beginning with the reducing group or potential aldehyde group in an aldose as No. 1, this will facilitate our reference to the various structures which apply to the known disaccharides. It must be remarked that the hydroxyl group attached to No. 1 position is located below the plane of the ring in the α-form and above the plane of the ring in the β-form and these two formulae are illustrated above. When we come to consider the mode of assembly of the pairs of glucose residues which occur in the representative bioses such as maltose and cellobiose, it is found that a unit of α-glucopyranose is linked with the hydroxyl at the 4th position of another glucose residue to give maltose, but on the other hand cellobiose is found to be derived from β-glucopyranose which is linked to a similar unit at the same 4th position.



Union of two α-Gluco-pyranose molecules

α-Maltose

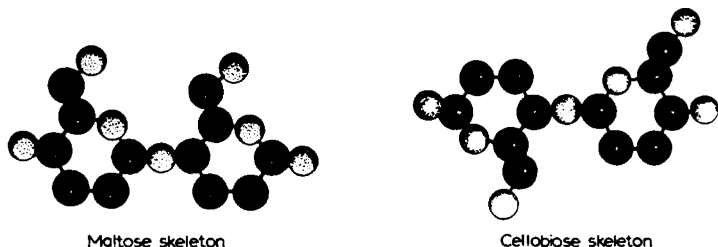
Union of two β-Gluco-pyranose molecules

β-Cellobiose

This is illustrated in the formulae shown above. In the case of cellobiose it is seen that the active group at No. 1 position of one glucose unit is above the plane, and is united to a hydroxyl group below the plane of the ring in the second residue at position 4. To bring the rings into alignment in the final cellobiose formula shown on the right, one of these rings is now in-

verted or turned through 180°. Although the units participating in the union of maltose are structurally identical with those assembled in cellobiose yet these products are widely different in kind.

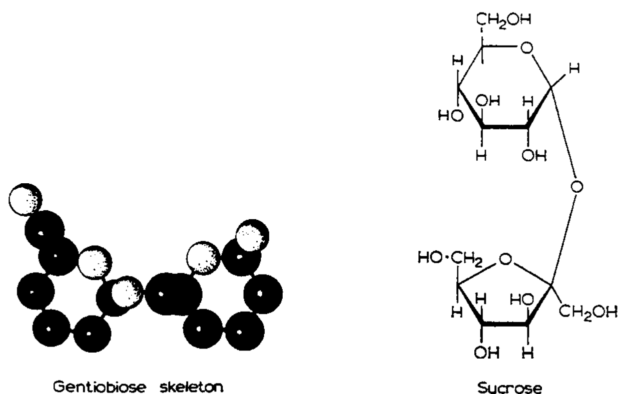
The difference lies entirely in the spatial arrangement of the left-hand formula indicating the α- or β-form of glucopyranose. This simple distinction furnishes the reason for the different entities of maltose and cellobiose.



Maltose skeleton Cellobiose skeleton

This difference is fundamental and provides also a reason for the difference in identity of starch and cellulose. It is found that starch is based entirely on the maltose model inasmuch as maltose is obtained in high yield from starch. On the other hand cellobiose is the representative disaccharide derived from cellulose and it is the mode of linking obtaining in cellobiose which is repeated throughout the whole molecule of cellulose. These constitutional forms are seen more clearly in the atom models which I represent as the skeletons of maltose and cellobiose.

Other disaccharides exist in which the linking is different. For example, the mode of assembly of two glucose units combined through the hydroxyl positions at 1 and 6 is found to occur in gentiobiose, which is the biose present in the glucoside amygdalin. In the examples given we have seen that the assembly of pairs of hexose units is effected by the linking of the hydroxyl at position 1 of one unit with the hydroxyl in another unit at either position 4 or 6. Turning now to the important disaccharide, sucrose, we fmd that here two different hexose units are involved, namely, glucose and fructose, which are assembled as a pair through the union of the hydroxyl at position 1 in glucose with that at No. 2, which is the reducing position in this case, in the fructose residue. Sucrose is unique in its constitution inasmuch as in this example of a biose we find a pyranose, or six-atom ring form, linked with a furanose, or five-atom ring form, and as the reducing positions in each are utilized in their combination to a biose, sucrose becomes a non-reducing sugar. Probably for this reason it is more easily crystallized

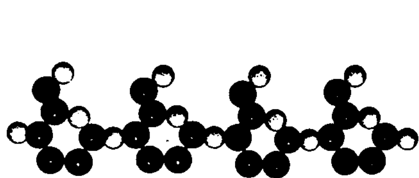


Gentiobiose skeleton

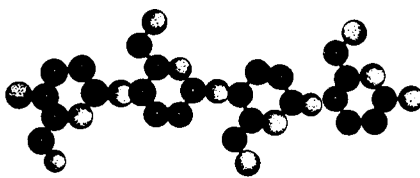
Sucrose

than the other bioses and we know it to be the most abundant, or the most easily available, of all pure organic substances.

From this rapid review of structural forms and of the modes of assembly of pairs of sugar units in a biose, let us now turn to illustrate similar experimental conclusions as to how more than two sugar molecules are assembled in various forms of carbohydrates. These formulations are based on the same kind of chemical proof as I have applied to the disaccharides, but in a review of this kind I propose to concern myself with results rather than with methods. Let us now take two pairs of maltose molecules and show how these are assembled in starch and glycogen and similarly how two cellobiose units are united as they appear in the cellulose chain. Thus we are able to approach to the constitutional picture representing starch and cellulose. Or if we envisage the procedure of adding repeating units of maltose to an ever lengthening chain we arrive at the model of starch and, by repeating a similar procedure with cellobiose, we represent repeating units as they occur in a very extended chain molecule in cellulose. Remarkable as the statement may appear it is nevertheless the case that these two models are structurally identical. They owe their differences to the two stereochemical forms of the same



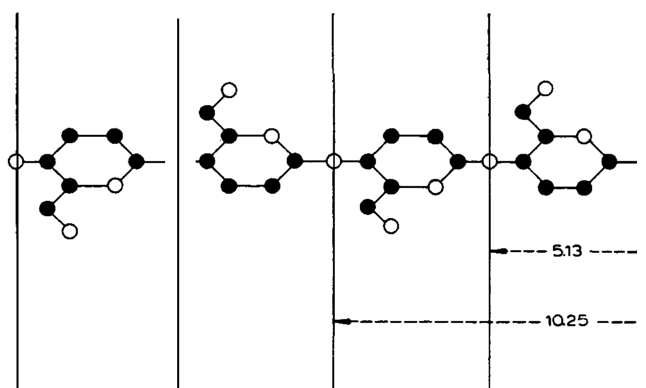
Two maltose units assembled as in starch



Two cellobiose units assembled as in cellulose

glucopyranose unit; as already stated, these are found to be α-glucopyranose in maltose and β-glucopyranose in cellobiose. The more symmetrical figure is that provided by the repeating pattern of cellobiose units. Here the side chain on each hexose appears alternately above and below in the picture, and this contrasts with the model shown for starch inasmuch as the side chain or 6th carbon atom occurs entirely on one side, a representation less symmetrical than that of cellulose. Moreover, the departure from the true linear arrangement is greater in the case of starch than of cellulose. The reason for this is apparent because in cellulose each alternate glucose unit is reversed and therefore any departure from the true linear direction is corrected in this model. We see therefore that in the structural representation for cellulose we have a reason for the occurrence of cellulose as a fibre in that its molecule approaches the rectilinear condition. Thus its conformation is more regular and provides a pattern which characterizes the X-ray diagrams for this substance. The diagram for starch is much less regular and less easy to interpret. Moreover there are other characteristic differences probably traceable to the continuous pattern formed by the assembly of α-glucose units in starch and to this we shall return. The cellobiose picture as determined by the classical methods of organic chemistry fits perfectly into the size of cell demanded by the X-ray diagrams, which fulfil every dimension of the repeating pattern of the cellobiose formula.

A question of particular moment is concerned with the length of the chain of repeating units in starch, cellulose, and glycogen. For it may now be said that glycogen has the same internal constitution as starch. On the other hand xylan or wood gum is constituted on a similar plan to cellulose except that it is built up of xylose units, largely, in place of glucose. Wisdom dictates



that in a problem of this complexity all the available polysaccharides should be studied together as a group. Information which may not be readily available from the study of one polysaccharide may be revealed by the study of another, and it is probable that a conclusion will be reached which is common to all of them. It is a reasonable supposition that Nature in building up polysaccharides follows a common plan. For this reason I have developed what is now known as the end-group assay of methylated polysaccharides as a preliminary to the study of the chain length. Unless these chains are constituted as continuous loops then there must be a terminal group which carries one more non-reducing hydroxyl than any of the intermediate units in the chain, or than the remaining end group which will terminate with a reducing unit. Our study of xylan has been important from this point of view. In xylan some 17 or 18 β-xylopyranose units are assembled in a chain which is terminated by one unit of arabofuranose. This latter can be easily removed by hydrolysis and there remains only a chain of xylose residues. In 1934 I pointed out that this picture of xylan was probably typical of other polysaccharides in that these chains of limited length aggregated to form a larger entity and the nature of the bonds effecting the union of adjacent chains was discussed. It was suggested that these might be either united by principal valency links or by some other type of bond such as that which is responsible for coordination. Whatever this kind of agency or link may be, I prefer to describe it as the polymeric bond and as such it may differ from ordinary valency bonds and may find currency in the whole field of polymeric substances.

In starch, for example, the individual chains terminate after 26 or 30 α-glucopyranose units and the chains are assembled by the same aggregative force as that just mentioned. It has been found possible to effect the reverse change of disaggregation in the case of starch. This was effected by mild acid treatment of the starch grains followed by acetylation and methylation. Very recently this observation has been confirmed by my former colleague E. L. Hirst who has isolated the methylated form of a single chain of 26 α-glucopyranose units. In the case of glycogen the chains differ from starch in being shorter in length and we have examined specimens of glycogen which contain continuous chains of both 12 and 18 a-glucose units. These chains again are interlinked by the polymeric bond to form a very large molecular complex showing a molecular weight of 1,000,000 or more.

The same experimental methods have been applied in order to gain an insight into the molecular size of cellulose. Here the complexity of the prob-

lem is very great. In 1932 I showed that, taking every precaution to avoid breakdown of cellulose, cotton linters could be acetylated under mild conditions and then methylated, by two treatments only with the reagents, to attain an almost completely substituted methylated specimen. This, by the method of end-group assay, contained one end group recognized as crystalline tetramethyl glucose for every 190 units of trimethyl glucose. The observation was made that the value of 100 to 200 β-glucopyranose units probably constituted the minimum length of chain in cellulose but that native cellulose untreated by chemical reagents would probably be found to possess still greater complexity. In this connection I suggested in 1935 that the molecular aggregate of cellulose may comprise an aggregation which not only increases the length of the chain, but also the width, by the lateral combination of adjacent chains. I pointed out that these factors must be recognized in any comparison of the molecular weight of cellulose determined by physical and chemical methods. All recent experiments in my laboratory have fully confirmed these conclusions. There can be no doubt that those forces which I describe as polymeric bonds are active in linking together adjacent chains of cellulose as in the case of xylan, glycogen, and starch. I do not share the view recently expressed that cellulose is constituted on the plan of a continuous loop of glucose units, this single loop being of a size to correspond with the high molecular weight found for cellulose by physical methods, although in my book on the constitution of sugars published in 1929 I suggested that this conception must be fully explored.

Time does not permit me to outline the range of facts which have been accumulated from our study of other polysaccharides such as inulin, mannan, and certain of the vegetable gums such as gum arabic, or gum acacia. But these experiments have thrown further light on the general problem of the molecular structure of complex carbohydrates.

Now, if I may, I should like to turn to another aspect of the subject of carbohydrates which brings us to the study of the constitution and synthesis of vitamin C.

The constitution and synthesis of vitamin C

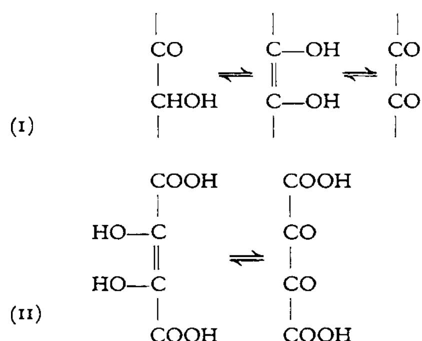
It will be recalled that in the course of his researches in 1928 Szent-Györgyi isolated from the adrenal cortex and also from orange juice and cabbage juice, a highly reducing substance which has many of the properties of a

carbohydrate. This substance had been named hexuronic acid in virtue of its acidity and strong resemblance to highly reactive sugar acids. It was crystalline, having melting point 192° and $[\alpha]_D$ + 23° in water; it was easily affected by oxidizing agents, and was capable of undergoing reversible oxidation by iodine or phenol indophenol. The molecular formula was established as $C_6H_8O_6$, and before any question of its relationship to a vitamin arose, Professor Szent-Györgyi paid me a visit in the University of Birmingham and invited me to investigate the constitution of this highly interesting substance.

Soon its possible connection with vitamin C appeared probable from the experiments of Szent-Györgyi and Svirbely and of Tillmans and also of Waugh and King. At this stage larger quantities of the material were prepared by Szent-Györgyi, first of all from adrenal cortex and later from Hungarian paprika, a richer and much more convenient source. On the suggestion of Professor Szent-Györgyi and myself, the name of the substance was changed from that of hexuronic acid, which was not distinctive, to that of ascorbic acid. It had been shown by Szent-Györgyi that the antiscorbutic activity was due to the substance itself and not to a contamination of the material with some more potent substance. Moreover, we prepared the primary oxidation product of ascorbic acid and this was found by Hirst and Zilva to be as active physiologically as the original ascorbic acid; and further, it was shown that the acid regenerated by the reduction of the oxidized material was still fully active. These observations showed that Tillmans' hypothesis concerning the reversible oxidation of vitamin C was indeed correct and they served also to explain the earlier observations of Zilva on the so-called "reducing factor". Further evidence that ascorbic acid was identical with vitamin C came from investigation of the potency of samples prepared from different sources; and the observation that synthetic ascorbic acid prepared from completely inactive materials had the same degree of physiological activity as the natural substance, furnished final and incontrovertible proof.

Constitution of ascorbic acid. Ascorbic acid is a monobasic acid, giving well-defined salts of the type $C_6H_7O_6M$. It is a powerful reducing agent and its oxidation can be effected in stages, the first of which requires the equivalent of one atomic proportion of oxygen for each molecule of ascorbic acid. When oxidation is arrested at this stage the product can be reduced quantitatively to ascorbic acid by reducing agents such as hydriodic acid or hydro-

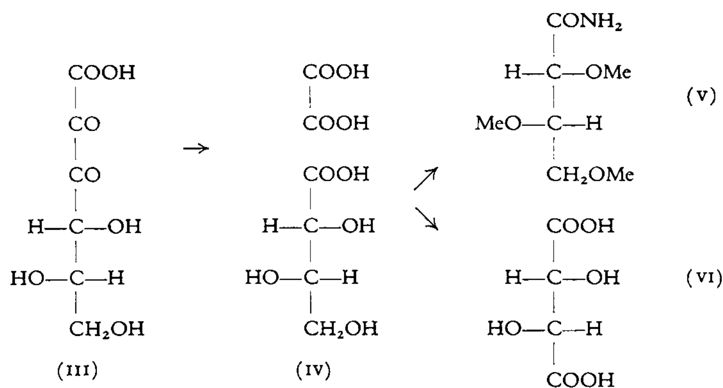
gen sulphide. Ascorbic acid is specially sensitive to oxidation by gaseous oxygen in the presence of minute traces of copper as catalyst, but in these circumstances the reaction proceeds beyond the reversible stage and involves destruction of the molecule. Ascorbic acid reacts readily with phenylhydrazine giving a product having the composition of an osazone. The presence of at least one keto group capable of undergoing enolization is thus confirmed and the character of the ultraviolet absorption spectrum (intense band at λ 245 mμ in acid solution) is in full agreement with this. Furthermore, the intensity of the absorption suggests that conjugated double bonds are present in the molecule. The above mentioned properties pointed to the presence in ascorbic acid of the group (I) and an exact analogy is provided by dihydroxymaleic acid (II) which displays absorption similar to that of ascorbic acid and undergoes reversible oxidation by iodine in acid solution.



Another property of ascorbic acid which was known at an early stage in the investigation and played an important part in the elucidation of the molecular structure was the unusual flatness of the molecule revealed by crystallographic and X-ray examination carried out in my laboratory by Dr. Cox. By attention to this criterion a choice could be made between alternative structural formulae which, at the commencement of the investigation, appeared to satisfy the requirements of the known chemical transformations.

Our observation that ascorbic acid could be transformed almost quantitatively into furfural provided strong evidence that the molecule contained a straight chain and not a branched chain of carbon atoms. Further evidence of this, and insight into the stereochemical relationships of ascorbic acid were obtained from a study of the oxidation, by sodium hypoiodite, of the primary (reversible) oxidation product. Two substances were obtained in al-

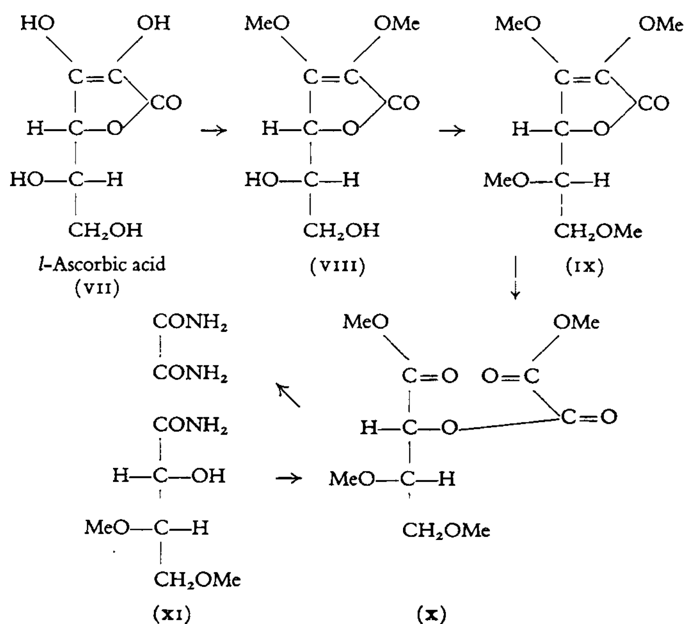
most quantitative yield, namely oxalic acid and *l*-threonic acid (IV), the identity of the latter being established by its transformation into trimethyl *l*-threonamide (V) and into *d*-tartaric acid (VI). These facts establish the conclusions that in alkaline solution the primary oxidation product of ascorbic acid reacts as a salt of the acid (I I I) and that ascorbic acid is related stereochemically to *l*-gulose



An important observation was that, when newly formed, the primary reversible oxidation product from ascorbic acid does not possess acidic properties but behaves in all respects as a lactone, which develops acidity when kept in aqueous solution. It followed that the acidic character of ascorbic acid is due to an enolic hydroxyl group and not to a free carboxyl group and, in order to determine the structure of ascorbic acid, it remained only to discover the nature of the lactone ring in the primary oxidation product. The main features of the constitution of ascorbic acid were now established and its formulation as a lactone of 2-keto-*l*-gulonic acid, capable of reacting in various tautomeric modifications, was first announced from the University of Birmingham, early in 1933.

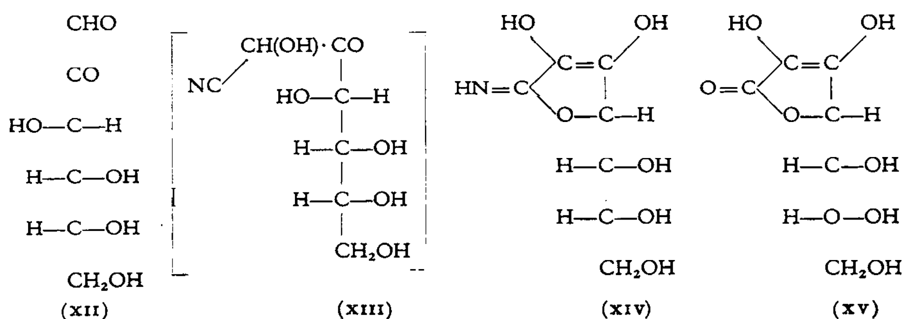
The elucidation of the nature of the lactone ring followed from a study of the oxidation product obtained when tetramethylascorbic acid reacts with ozone. It had been shown by Micheel that the dimethyl derivative of ascorbic acid, obtained by Karrer by the action of diazomethane, gives a di-*p*-nitrobenzoyl derivative and that the latter reacts with ozone giving a neutral ester containing the same number of carbon atoms as the unoxidized material. It followed that a ring system must be present in ascorbic acid, but the products obtained on hydrolysis of the neutral ester (oxalic acid and *l*-threonic acid) did not permit of deductions being made concerning the na-

ture of the ring, and the interpretation at that time advocated by Micheel was invalid in that it involved the presence of a free carboxyl group in ascorbic acid. By reference to the accompanying formulae it will be seen that by application of a similar method of oxidation to fully methylated ascorbic acid the nature of the ring system can be determined with certainty. It was found that dimethylascorbic acid (VIII) was readily converted into the corresponding tetramethyl derivative (IX) by the action of silver oxide and methyl iodide, and that this on treatment with ozone gave rise to a neutral ester (X) which reacted with ammonia giving oxamide and the amide of 3:4-dimethyl-*l*-threonic acid (XI). The presence of a hydroxyl group in the α-position in the latter substance was proved by the observation that the amide gave a strong positive Weerman reaction (formation of sodium cyanate by the action of sodium hypochlorite on the amide). It follows immediately that in ascorbic acid, in dimethylascorbic acid, and in tetramethyl ascorbic acid, the lactone ring is of the γ-type and engages the hydroxyl group attached to the fourth carbon atom of the chain. Ascorbic acid is therefore to be represented by (VII). If, on the other hand, ascorbic acid had contained a δ-lactone ring the products, obtained by the action of ammonia on the neutral ester formed by ozonization, would have been oxamide and 2:4-dimethyl-*l*-threonamide and the latter amide would not have under-

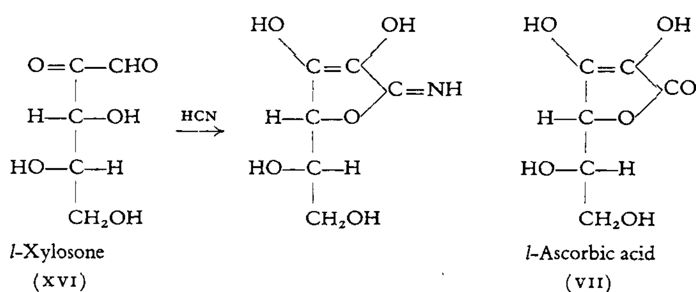


gone the Weerman reaction. The structure (VII) (enolic form of 2-keto-*l*-gulonolactone) is in full agreement with all the chemical properties of ascorbic acid and, when an atomic model of this constitution is built up, it is seen that the structure is almost flat and its dimensions account satisfactorily for the crystallographic and X-ray observations.

The synthesis of ascorbic acid. The two principal methods which are now available for the synthesis of ascorbic acid and its analogues are: (a) addition of hydrogen cyanide to an osone followed by acid hydrolysis of the addition compound, and (b) the re-arrangement of 2-keto-3:4-dihydroxy-acids or their esters. Method (a) suffers from the disadvantage that it requires osones as starting materials, and when these are available it is a powerful and certain method which has been utilized for the preparation of many analogues of ascorbic acid. Moreover, it was the method employed simultaneously by Reichstein and by Hirst and myself, in the first synthesis of the *d*- and *l*-isomerides of ascorbic acid. The mechanism of the reaction has been the subject of detailed investigation and it will be illustrated by reference to the synthesis of *d*-gluco-ascorbic acid. The first stage of the synthesis from glucosone (XII) results in the formation of $C_7H_{11}O_6N$, a crystalline addition product which displays a strong absorption band at λ 275 mμ. The properties of this substance show that it is not the open-chain cyanohydrin (XIII) but the cyclic imino-compound (XIV) which exists in aqueous solution as a neutral internal salt, evidence on this point being obtained from studies of the optical rotatory dispersion of the substance in neutral and in acid solution (when the ionisation is suppressed). Similar cyclic bodies have been obtained in the course of synthesis of other analogues of ascorbic acid and the reaction appears to be a general one. The intermediate cyclic-imino body (XIV) possesses many of the characteristic properties of ascorbic acid (e.g. intense



absorption band, oxidation by iodine in acid solution) and on hydrolysis by aqueous acid the imino group is removed and gluco-ascorbic acid (XV) is produced. The latter substance possesses the characteristic ring system of ascorbic acid, and displays chemical properties (and an absorption spectrum) closely similar to those of natural ascorbic acid. The synthesis of natural *l*-ascorbic acid (vitamin C) proceeds in a similar way from *l*-xylosone (XVI) which is obtainable from *d*-galactose as the outcome of the following series of transformations : *d*-galactose→ *d*-galactose-*1:2,3*:4-diacetone → *d*-galacturonic acid-1:2,3:4-diacetone → *d*-galacturonic acid → *l*-galactonic acid → *l*-galactonamide→ *l*-lyxose→ *l*-xylosazone→ *l*-xylosone

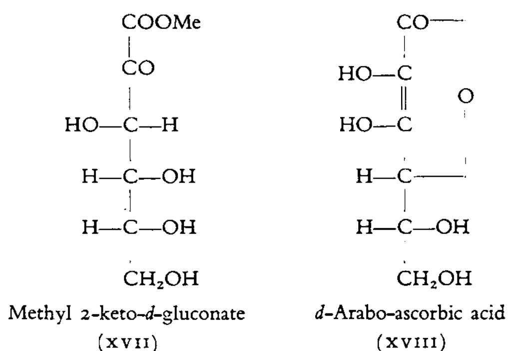


l-Xylosone (XVI)

l-Ascorbic acid (VII)

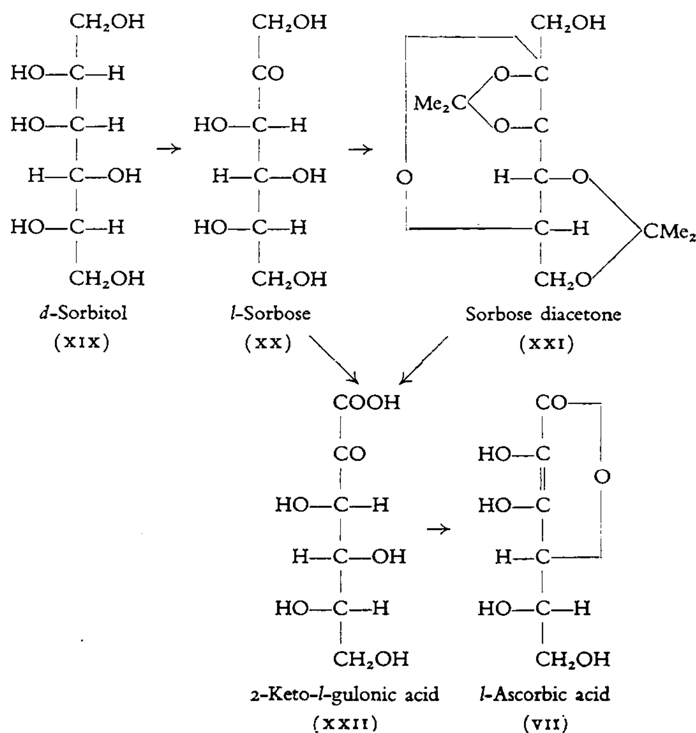
Several analogues of *l*-ascorbic acid have also been synthesized by the above-mentioned method. It may be observed that the nomenclature adopted for convenience of reference derives the name of the substance from that of the osone used in the synthesis.

In the preparation of *l*-ascorbic acid it is advantageous to avoid the use of *l*-xylosone which is not easily accessible. By application of the second mode of synthesis notable advances have been made in the ease of preparation of synthetic vitamin C. This method was first applied by H. Ohle in the preparation of *d*-arabo-ascorbic acid (XVIII) from 2-keto-*d*-gluconic acid. The acid, and more particularly the methyl ester (XVI I), undergoes ring closure and isomerization under a variety of conditions, amongst which the action of sodium methoxide may be referred to as of special importance, and the resulting substance possesses the ring system of ascorbic acid. In the case of natural ascorbic acid the necessary keto-acid is 2-keto-*l*-gulonic acid (XXII) which can be readily obtained, as shown by Reichstein, from *l*-sorbose (XX) by oxidation of sorbose diacetone (XXI) and subsequent removal of the acetone residues. *l*-Sorbose in turn is now available in quantity by the bacterial oxidation of *d*-sorbitol (XIX).



Methyl 2-keto-*d*-gluconate (XVII) *d*-Arabo-ascorbic acid (XVIII)

A still simpler method for the synthesis of *l*-ascorbic acid consists, as I have shown by my experiments, in the direct oxidation of *l*-sorbose which like *d*-fructose, is specially sensitive to oxidation at the primary alcoholic group at C_1. When oxidized under carefully controlled conditions by nitric acid *l*-sorbose (XX) is transformed directly into (XXII), and the methyl ester of (XXII) gives the sodium salt of *l*-ascorbic acid when treated with sodium methoxide. In a similar way *d*-fructose gives rise to 2-keto-*d*-glu-



d-Sorbitol (XIX) *l*-Sorbose (XX) Sorbose diacetone (XXI)

2-Keto-*l*-gulonic acid (XXII) *l*-Ascorbic acid (VII)

conic acid, the methyl ester of which yields the sodium salt of *d*-arabo-ascorbic acid (XVI II) on treatment with sodium methoxide.

That the distinguishing feature of ascorbic acid and its analogues, in so far as absorption spectra and chemical properties are concerned, lies in the enolic double bond, may be illustrated by reference to the work of H. von Euler and C. Martius on reductone (hydroxymethylglyoxal, CHO.C(OH) =CHOH) which resembles ascorbic acid chemically and in its absorption of light, and is strongly acidic without possessing a carboxyl group (Norrish and Griffiths).