Chemistry 1937

WALTER NORMAN HAWORTH

<<for his investigations on carbohydrates and vitamin C>>

PAUL KARRER

<<for his investigations on carotenoids, fIavins and vitamins A and B_2>>

Chemistry 1937

Presentation Speech by Professor W. Palmaer, Chairman of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesty, Royal Highnesses, Ladies and Gentlemen.

To the most important chemical compounds belongs a group of substances named carbohydrates. They have been so called because of their composition, which is such that they may be considered as built up by a combination of carbon and water-hydrates is the common terminology for chemical compounds in which water is a constituent. The least complicated forms of carbohydrates are the so-called monosaccharides, among which belongs grape-sugar, so designated because of its presence in the juice of grapes. The Latin name of sugar is *saccharum;* hence, the various types of sugar are also named saccharides. By a combination of particles-molecules- from the monosaccharides under separation from part of the water, complex saccharides are obtained, in the first place disaccharides, among which may be mentioned the well-known cane-sugar, and also milk-sugar and maltose. By continued combination more complex carbohydrates may be obtained, which have too been called polysaccharides, even though they have lost the properties of sweet taste and easy solubility in water. To these compounds belong, i.a., all kinds of starch, which constitute such an important part of our food, and also *cellulose,* the building material of the plants, which represents the most complicated form of the carbohydrates. While one molecule of grape-sugar is built up by *6* atoms of carbon, 12 of hydrogen, and 6 of oxygen, the number of atoms in one molecule of cellulose probably exceeds two thousand.

When the Nobel Prize in Chemistry was distributed for the second time, in the year 1902, it was awarded to the scientist of genius Emil Fischer, in recognition of his investigations partly concerning sugars and partly relating to caffeine and substances allied thereto.

This year the Royal Academy of Sciences has decided to attribute one half of the Nobel Prize in Chemistry to Professor W. N. Haworth of Birmingham in recognition of his researches concerning carbohydrates and vitamin C.

One may perhaps question if there remained much to be done within the domain of chemistry of the carbohydrates after the classical works of Emil

Fischer. This question must, however, be answered in the affirmative. To take only the case of monosaccharides of the type of grape-sugar, no less than 32 different forms are possible, all having the same chemical composition and containing an equal number of atoms in the molecule, but still differing from each other, this difference depending on the different arrangement of the atoms within the molecule. The possibilities are still more diverse when complex saccharides - the disaccharides - are considered, to say nothing of starch and cellulose, and these differences, in the case of the saccharides alone, small as they may appear, are yet of great interest, not only from a theoretical point of view, but also for the comprehension of the central role of the sugars in metabolism, as well as in their technical applications.

It is true that Haworth is not alone in having effected progress within this domain. He commenced his researches with his countryman Irvine, who has also produced eminent works relating to carbohydrates. Others, too, among them the Nobel Laureate in Chemistry this year, Professor Karrer, have made highly meritorious contributions. Among the works of Haworth, his researches regarding the different forms of grape-sugar and on the arrangement of the atoms in cane-sugar, maltose and milk-sugar, starch and cellulose, ought to be given special prominence.

Among the motives for the awarding of the prize are also mentioned, however, the researches upon vitamin C which have been made by this scientist and which stand in close relation to his work on the monosaccharides.

The vitamins represent substances which have lately attracted the greatest interest and about which, up till very few years ago, the public knew just as much - or as little - as the chemists. It had been found that certain mysterious substances were necessary, though only in very diminutive quantities, in connection with the foodstuffs proper - i.e. carbohydrates, fats, and proteins - together with certain mineral salts, for the growth and normal development of the animal body, and that a lack of these substances caused diseases of various kinds. The animal body itself, in general, is lacking in the capacity to produce these substances, which must therefore be supplied in a ready form from vegetables, or else be prepared within the body from other more complicated substances contained in the vegetable foodstuffs.

The discovery of the vitamins has already been honoured by the awarding of Nobel Prize in Medicine. In 1929 one half of such a prize was awarded to the Dutchman Eijkman in recognition of his discovery that the eating of polished or peeled rice produced the severe, chiefly tropical, disease called

beri-beri, while people eating unpeeled rice remained quite sound. Hence he reached the conclusion that a substance of the kind aforesaid, now described as the antineuritic vitamin, or vitamin B_2, was contained in the husk or peel of the rice. The other half of the Nobel Prize in Medicine was awarded in the same year to Hopkins in recognition of his discovery of the vitamins of growth, that is, the substances necessary for the growth of the animal body - contained for instance in milk - and of which one of the most important has now been identified with vitamin A. And today the Nobel Laureate in Medicine is awarded his prize, i.a., for his discovery in connection with the very same vitamin which had been made the subject of investigation by Haworth.

What has Haworth then accomplished within this domain? The answer may be thus formulated that he has, above all, made clear the chemical structure of vitamin C.

The chemical structure of substances is expressed by the so-called chemical formulas. By chemical analysis the percentage of the different elements - in this case of carbon, hydrogen, and oxygen - which enter into a compound may be ascertained. Further, the weight of the atoms of the different elements, expressed for instance in relation to the atom of hydrogen, has long been known, the hydrogen atom being the lightest of all the elements. It is likewise possible to determine the weight of a particle, or molecule, of a compound, expressed in the same measure. It is hence possible to indicate how many atoms of the different elements are entering into one molecule of the compound. Thus, the gross formula of the compound is obtained. This formula, in the case of vitamin C, is quite simple, considering that it represents a vitamin, viz. : $C_6H_8O_6$ This formula tells us that one molecule of vitamin C consists of 6 atoms of carbon, 8 of hydrogen, and 6 of oxygen. It also indicates that vitamin C may be conceived as having originated through the elimination of 4 atoms of hydrogen from one molecule of grape-sugar.

But it is possible to advance still further. By ingenious adjustment or speculation, reminding us somewhat of the play of a puzzle, only perhaps a little more intricate, a firm conception has been formed about the order in which the atoms combine. If we conceive an ultra-enlarged model of a molecule, taken at a certain moment -because the atoms are not at a standstill within the molecule - and place a white screen on the one side of the model, while the other is exposed to light, a shadow-figure, also called a projection, of the molecule is obtained on the screen, showing the position

of the atoms in their relation to each other. A formula which is intended to reproduce this situation, under the assumption that the atoms were placed on the same plane, is called a structural formula. Such formulas have proved capable of explaining with a high degree of clarity the properties of the compound, and the puzzle thus may be considered as having been solved.

In reality it is, however, hardly correct to suppose that all the atoms within a molecule should be placed on the same plane; if that were the case, even the largest molecules would have the shape of a leaf of paper, which is less than probable. There remains then their dispersion in space, the so-called configuration, which also may be expressed by a formula.

Such a formula for vitamin C has been proposed by Haworth and Hirst, as well as by von Euler and has been subsequently proved to be correct by Haworth.

Before entering upon the practical significance of knowing the chemical structure of a vitamin, I ought to say a word about the notable properties which characterize vitamin C -the terminology does not, of course, give any indication in this regard. But this vitamin was previously called the antiscorbutic vitamin on the ground that the lack thereof caused the disease of scurvy, so much dreaded by the polar explorers of earlier times. This disease appeared during periods when the members of these expeditions were compelled to live on badly preserved foodstuffs, whereas the danger has been obviated by the introduction of better food preservation and a supply of fresh vegetables. The chemical name is ascorbic acid. This indicates on the one hand that the substance is an acid, on the other hand that it has a counteracting effect on scurvy, the medical name of which is *scorbutus;* thus the word ascorbic acid is equivalent to anti-scurvy acid.

The knowledge regarding the constitution of a vitamin does not only possess a theoretical interest but is also of very great practical importance. On the one hand it may be found possible, by minor changes in the known composition which may be brought about in an artificial way, to produce compounds which in some cases may prove to be more suitable as medicine. And above all, it opens the way to the artificial production of the compound, a thing of very great importance in the case of vitamins which do occur in nature only in a state of very great dilution. Thus vitamin C is already produced on a technical scale and at a price very much lower than that of the natural product.

The Royal Academy of Sciences has decided also to award to Professor Paul Karrer in Zurich one half of the Nobel Prize in Chemistry this year

in recognition of his researches concerning carotenoids and flavins, and the vitamins A and B2.

Thus these two scientists have both worked on another common field of research, the vitamins. As I have already endeavoured to elucidate at some length the importance of making clear the chemical structure of the vitamins, taking vitamin C as an example, I may be somewhat brief regarding the brilliant discoveries made by Professor Kaner.

The carotenoids form a group of yellowish-red colouring matters, widely dispersed within the vegetable kingdom, which have obtained their name from the carrot in which they were first observed. The French name of the carrot is known to be carotre, while Kurotfe is one of the German names thereof. Carotenoids occur in various other red or yellow parts of vegetables, such as tomatoes, hips, turnips. The examination of these numerous substances was commenced by Karrer ten years ago, and he has succeeded in making clear their chemical structure. The mother substance is in itself a hydrocarbon of very complicated composition, i.e. a chemical compound consisting only of carbon and hydrogen. Its molecule consists of no less than 40 atoms of carbon and 56 of hydrogen. Other carotenoids also contain oxygen, as is the case, for instance, with astacene, which gives the red colour to boiled crayfish and to the <<cardinal of the sea>>, the lobster. The colour of saffron and of paprica is likewise due to carotenoids.

The splendid research concerning the carotenoids, made by Karrer, received its coronation, when it led to the isolation, the production in a pure form and the determination of the chemical structure of vitamin A. This vitamin, which had been known to exist from its biological effects already since 1906 and the synthesis of which in a pure form had been tried in vain in many laboratories all over the world, was successfully isolated by Karrer in 1931 from cod-liver oil, and it was the first of the vitamins of which the chemical structure was clarified. It forms a growth factor, i.e. a substance necessary for the growth of the body. In 1929 von Euler found the same property existing in the carotine itself, and it has been proved since then that this is dependent on the circumstance that carotine, that is the dyestuff of the carrot, is a substance from which the animal body can in itself produce the vitamin A, which has a somewhat less complicated structure. It is also a medicine, as it prevents the serious disease of the eye called <<dry eye>> or xerophthalmia. Hence vitamin A has received the name of axerophthol.

Some words now regarding Karrer's researches on flavins and on vitamin B_2, which were commenced in 1933. Flavins are natural substances of a light

yellow colour which often glisten, or fluoresce to the green. One of them is vitamin B_2, also called lactoflavin, which was discovered by Warburg and Christian in the yellow respiratory ferment, and which has also been disentangled in regard to its chemical structure by Karrer. It constitutes likewise a growth factor, and Karrer's method of producing this compound has led to a technical production of the substance, which is of great biological importance. It contains, besides carbon, hydrogen and oxygen and also nitrogen.

Karrer has thus succeeded in elucidating completely the nature of two of the vitamins, hitherto considered as so mysterious, and one of them is now produced artificially. A characteristic of this scientist is his open eye to the great and important problems as well as to their kernels, and the independent way in which he attacks the problems and pursues his new departures with the aid of his own methods.

There remain many questions to be studied regarding the way in which the vitamins cooperate in such processes of life as cannot be started without their presence.

A vitamin does certainly not produce the effect alone, however. The lactoflavin, for instance, combines, with the aid of phosphoric acid, with an albuminous substance, and only in this way the yellow respiratory ferment is formed. Its molecule contains about 200 times as many atoms as that of the vitamin itself. The yellow ferment is reckoned as belonging to the catalyzers, i.e. substances capable to accelerate a chemical reaction without undergoing any change themselves. Their action may be compared to that of a lubricating oil on a rusty machine. In this case the oxidation of certain substances present in the body is taking place, thus a kind of combustion, although of course much slower than for instance the burning of wood in a stove. We may perhaps compare the very effect of the vitamin to that of a key. A heavy door may thus resist the strongest blows and knocks, but can easily be opened by the aid of a small key -always provided that the right key is found.

The discoveries, which have now engaged our attention, touch upon the domain of Physiology as well as that of Chemistry, a circumstance which has found its expression in that they have been awarded Nobel Prizes in Medicine as well as in Chemistry. Often it is just within the borderland between two sciences, where efforts have been frequently made to establish demarcatory lines (‘although mostly in vain), that the important discoveries are to be found. In such cases it is evidently of small avail, generally speaking,

to try to decide, even with the aid of the greatest acuteness, to which field of science such discovery should be properly attributed. The principal thing is, however, that the discoveries are recognized, if such he their value, and the classification of the prize awarded is a question of minor importance. In the present case it may be said, nevertheless, that the discoveries which have been awarded a prize in Chemistry are on the whole more chemically accentuated in their character than those which have received the prize in Medicine. In all the cases, however, such discoveries may be said to have <<conferred the greatest benefit on mankind>> in accordance with the intentions expressed in the will of Alfred Nobel.

Professor Haworth. The Royal Swedish Academy of Sciences has resolved to adjudge to you and Professor Karrer this year's Nobel Prize in Chemistry in recognition, for your part, of your most important researches into carbohydrates through which another epoch in the chemistry of these substances has been completed, and for your investigation of the constitution of vitamin C which is now produced artificially.

It is with the most sincere gratification that I have the honour of conveying to you the congratulations of the Academy on this distinction and I request you now to receive the Prize from his Majesty the King who has been graciously pleased to consent to hand it over to you.

Professor Karrer. The Royal Swedish Academy of Sciences has decided to confer upon you and Professor Haworth this year's Nobel Prize in Chemistry. In this way the Academy wishes to express to you her recognition for your brilliant investigations on carotenoids and flavins, as well as on vitamins A and B,. As a result of your work, the structure of a vitamin has for the first time been clarified. The structure of a second vitamin has also been cleared up, thus enabling its technical preparation.

I convey to you the congratulations of the Academy and request you to receive the prize from the hands of his Majesty the King.

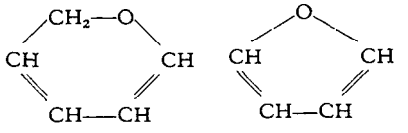
WALTER N. HAWORTH

The structure of carbohydrates and of vitamin C

Nobel Lecture, December 11, 1937

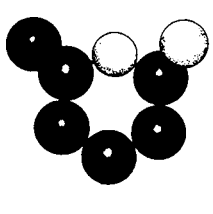
The structure of carbohydrates ad of vitamin C

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.

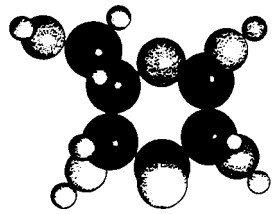


Pyran Furan

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–

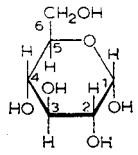


Skeleton model of glucose

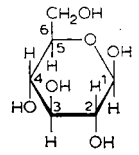


Model of β-glucose

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



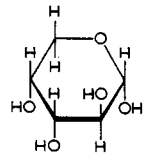
α–Glucopyranose

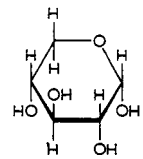


β–Glucopyranose

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.

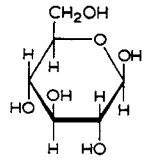
α-Arabo-pyranose

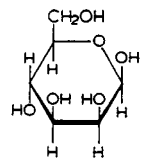




α-Xylo-pyranose

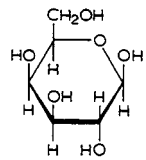
β-Gluco-pyranose

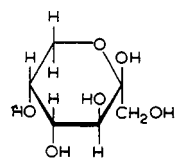




β-Manno-pyranose

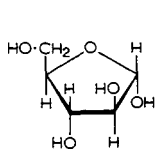
β-Galacto-pyranose



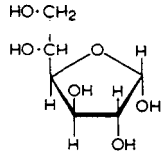


β-Fructo-pyranose

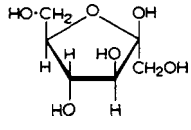
Much less stable forms of pentose and hexose are those which I have described as the furanose forms. Only the derivatives of these have been isolated as homogeneous crystalline substances, e.g. the ethyl- and methyl-glucosides which are hydrolysed with something of the order of 100 times the velocity 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.



α-Arabo-furanose



α-Gluco-furanose



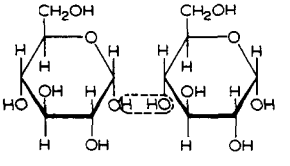
β-Fructo-furanose

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

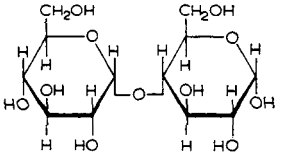
$$C_6H_{12}O_6 + C_6H_{12}O_6 = C_{12}H_{22}O_{11} + H_2O$$

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.

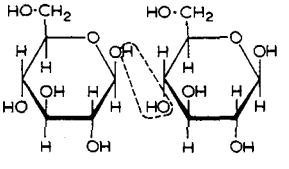
If we utilize a method of numbering the carbon atoms of a pyranose, beginning with the reducing group or potential aldehyde group in an aldose as No. 1, this will facilitate our reference to the various structures which apply to the known disaccharides. It must be remarked that the hydroxyl group attached to No. 1 position is located below the plane of the ring in the a-form and above the plane of the ring in the p-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.



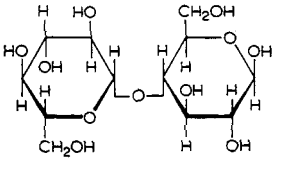
Union of two α–Glucopyranose molecules



α– Maltose



Union of two β–Glucopyranose molecules

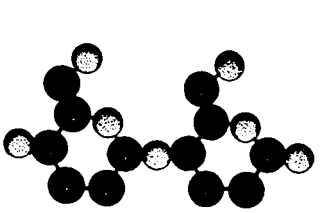


β –Cellobiose

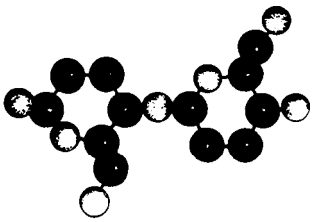
This is illustrated in the formulae shown above. In the case of cellobiose it is seen that the active group at No. 1 position of one glucose unit is above the plane, and is united to a hydroxyl group below the plane of the ring in the second residue at position4. To bring the rings into alignment in 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 b-form of glucopyranose. This simple distinction furnishes the reason for the different entities of maltose and cellobiose.



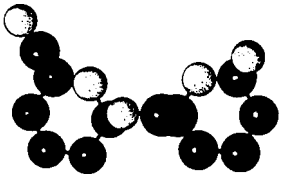
Maltose skeleton



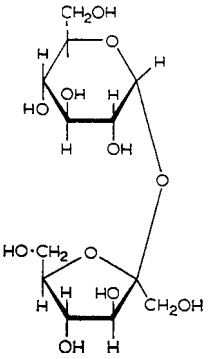
Cellobiose skeleton

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



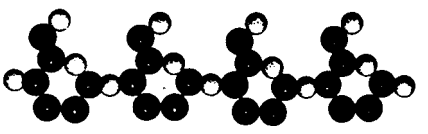
Gentiobiose skeleton



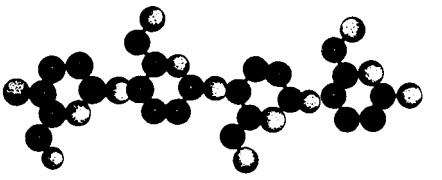
Sucrose

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



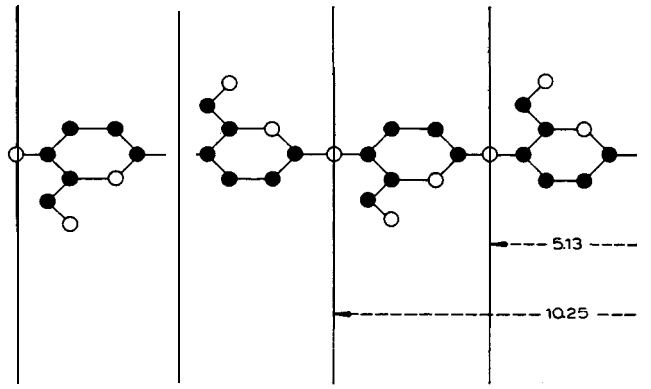
Two maltose units assembled as in starch



Two cellobiose units assembled as in cellulose

glucopyranose unit; as already stated, these are found to be a-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

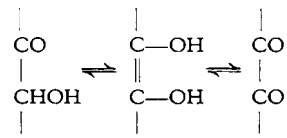
carbohydrate. This substance had been named hexuronic acid in virtue of its acidity and strong resemblance to highly reactive sugar acids. It was crystalline, having melting point 192° and $[\alpha]_D$ + 23° in water; it was easily affected by oxidizing agents, and was capable of undergoing reversible 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.

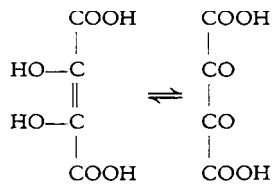
Constitution of ascorbic acid. Ascorbic acid is a monobasic acid, giving well-defined salts of the type $C_6H_7O_6M$. It is a powerful reducing agent and its oxidation can be effected in stages, the first of which requires the equivalent of one atomic proportion of oxygen for each molecule of ascorbic 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.

(I)



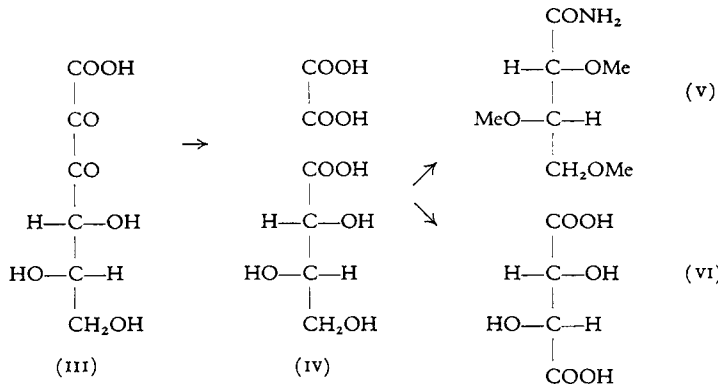
(II)



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-

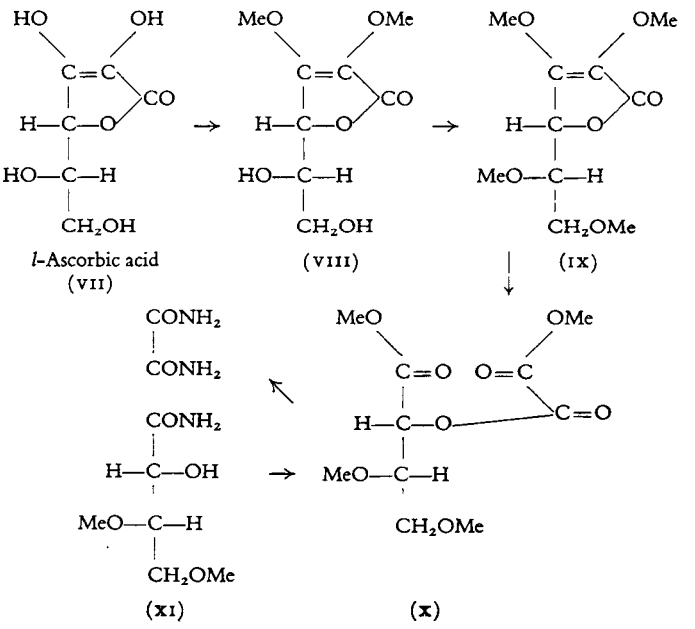
most quantitative yield, namely oxalic acid and *l-threonic* acid (IV), the identity of the latter being established by its transformation into trimethyl *l-threonamide* (V) and into *d-tartaric* acid (VI). These facts establish the conclusions that in alkaline solution the primary oxidation product 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.

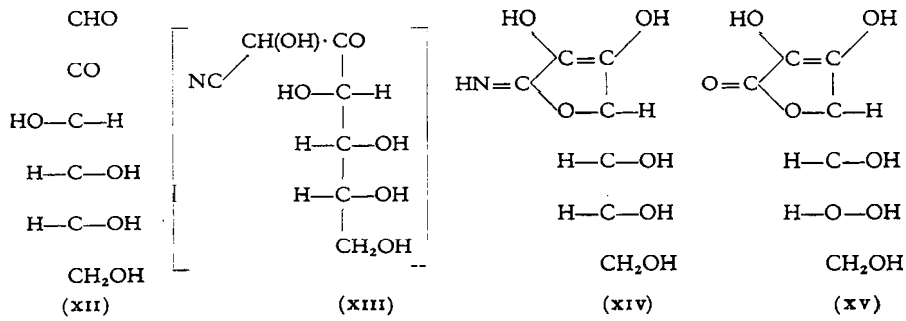
The elucidation of the nature of the lactone ring followed from a study of the oxidation product obtained when tetramethylascorbic acid reacts with ozone. It had been shown by Micheel that the dimethyl derivative of ascorbic acid, obtained by Karrer by the action of diazomethane, gives a di -*p*-nitrobenzoyl 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 *y*-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-

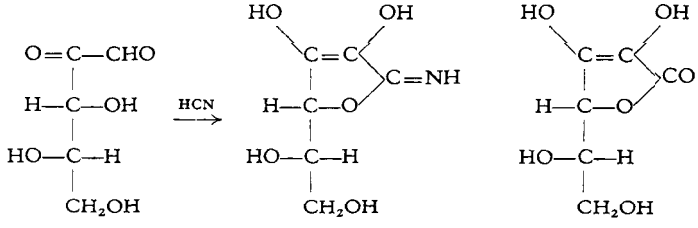


gone the Weerman reaction. The structure (VII) (enolic form of 2-keto-l-gulonolactone) is in full agreement with all the chemical properties of ascorbic acid and, when an atomic model of this constitution is built up, it is seen that the structure is almost flat and its dimensions account satisfactorily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



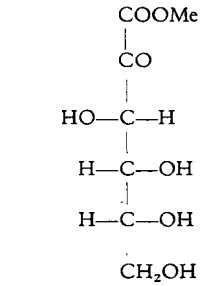
absorption band, oxidation by iodine in acid solution) and on hydrolysis by aqueous acid the imino group is removed and gluco-ascorbic acid (XV) is produced. The latter substance possesses the characteristic ring system of ascorbic acid, and displays chemical properties (and an absorption spectrum) closely 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*.



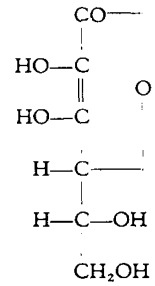
l-Xylosone (XVI) *l*-Ascorbic acid (VII)

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).

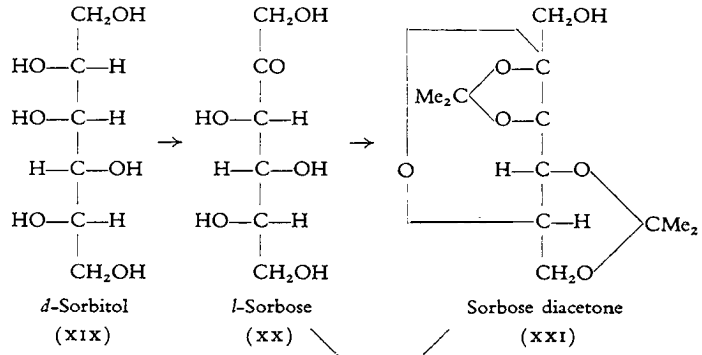


Methyl 2-keto-*d*-gluconate
(XVII)



d-Arabo-ascorbic acid
(XVIII)

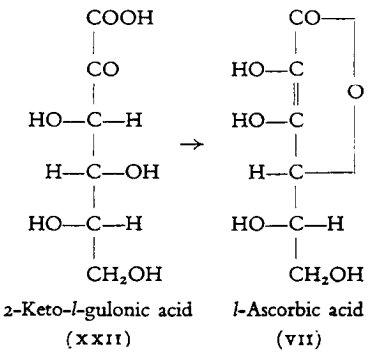
A still simpler method for the synthesis of I-ascorbic acid consists, as I have shown by my experiments, in the direct oxidation of *l-sorbose* which like d-fructose, is specially sensitive to oxidation at the primary alcoholic group at C_1. When oxidized under carefully controlled conditions by nitric acid *l-sorbose* (XX) is transformed directly into (XXII), and the methyl ester of (XXII) gives the sodium salt of I-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-as*-corbic 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) = C H O H) which resembles ascorbic acid chemically and in its absorption of light, and is strongly acidic without possessing a carboxyl group (Norrish and Griffiths).

Biography

Walter Norman Haworth was born at Chorley, Lancashire, on March 19, 1883. He attended the local school until the age of fourteen when he joined his father, Thomas Haworth, to learn linoleum design and manufacture. His interest in chemistry was aroused through the use of dyestuffs in his work and his thirst for further knowledge led him to seek private tuition in Preston. This coaching enabled him to pass the entrance examination of the University of Manchester and in 1903 he entered the Chemistry Department as a pupil of W. H.Perkin, Junior. He graduated with first class honours in 1906 and after three years research he went, on a scholarship, to Wallach's laboratory at Göttingen. He received his doctor's degree in 1910 and returned to Manchester to be awarded his D. Sc. degree in 1911-these qualifications were gained in the minimum time possible.

In 1911, Haworth took an appointment as a demonstrator at the Imperial College, London and in 1912 he moved to St. Andrews, Scotland, as Lecturer and Reader in Chemistry. In 1920, he was called to the Chair in Chemistry at the University of Durham and in the following year succeeded Phillips Bedson as Director. Haworth was appointed Professor and Director of the Department of Chemistry in the University of Birmingham in 1925 and he remained in this position until his retirement in 1948, becoming Dean of the Faculty of Science and acting as Vice-Principal during 1947-1948. Sir Norman was active in retirement, serving on many Boards and Committees; he represented the Royal Society at the Seventh Pacific Science Congress in New Zealand during February, 1949. He was knighted in 1947.

Haworth's early researches, initially with Perkin, involved investigations on the constitution of terpenes and in 1912 he synthesized sylvestrene. At St. Andrews, in association with T. Purdie and J. C. Irvine, he turned his attention to carbohydrates, extending Emil Fischer's method of reacting sugars with methanol to an elegant preparation of methylated derivatives which were, in turn, .used to characterize the constitution of sugars.

During the First World War, Haworth organized the laboratories at St. Andrews for the production of fine chemicals and drugs; after the war he

returned once more to his carbohydrate investigations. By 1928, he had evolved and confirmed, among others, the structures of maltose, cellobiose, lactose, gentiobiose, melibiose, gentianose, raffinose and the glucoside ring structure of normal sugars. He studied lactones from sugars and co-related structure with optical rotatory powers. His method for the determination of chain length in methylated polysaccharides, an important structural problem, helped to settle the basic features of the starch, cellulose, glycogen, inulin and xylan molecules.

Following the synthesis of ascorbic acid, which considerably cheapened commercial production, Haworth's later researches contributed greatly towards further co-ordination of the chemical, physical and biological, problems concerned with bacterial polysaccharides.

Haworth wrote numerous scientific papers and contributed to Advances in Carbohydrate Chemistry. His book The Constitution of Sugars was published in 1929.

Haworth was President of the Chemical Society (1944-1946), and Fellow (1928), and vice-President (1947-1948) of the Royal Society He received honorary science degrees from the Universities of Belfast, Zurich and Oslo, honorary Doctor of Law, University of Manchester, and foreign memberships of nine foreign scientific academies. He was the Longstaff Medallist (Chemical Society), 1933; Davy Medallist (Royal Society), 1934, and Royal Medallist, 1942.

In 1922, Haworth married Violet Chilton, second daughter of Sir James Dobbie, LL.D., F.R.S. They had two sons.

He died suddenly on March 19, 1950.

PAUL KARRER

Carotenoids, flavins and vitamin A and B_2

Nobel Lecture, December 11, 1937

In the last few decades chemical research has been divided in the main into two directions; one part aims at the investigation of the structure of the atom in the widest sense, the other at the elucidation of the composition of the living cell and its chemical reactions. The first direction has availed itself in many ways of entirely new methods of investigation, which are essentially of a physical nature. Biochemical research was able to base itself so far in the main on the methods of classical chemistry, which certainly have been improved and developed in an extraordinary manner.

Progress in methods which modern biochemistry can no longer dream of doing without, are the selective adsorption processes of Willstätter, separation by the ultracentrifuge of Svedberg, and chromatographic analysis by Tsvett, by the use of which it is now possible to separate mixtures of substances which were previously inextricable. Chromatographic analysis, in particular, which is based upon filtration of the solution of the mixture of substances through an adsorption column, in which the more easily adsorbed substances are retained in the upper part of the tube, with the substances more difficult to adsorb being retained in the lower part, operates in a similarly complete manner like a prism, which divides the white light into the individual colours of the spectrum. Thanks to these efficient separation processes our picture of the composition of plant and animal cells is today essentially more complicated than it was a few years ago, and it cannot be doubted that to later generations there will be revealed an even greater diversity and non-uniformity of the living cell.

The blue and red pigments of blossoms and berries, the anthocyanins, which were formerly thought to be uniform and characteristic for the various plants concerned, have been shown by later investigations to be mixtures of many closely related substances. There is indeed not one flower, not one berry, which owes its colouration to a single anthocyanin. Similar conditions apply in another group of natural pigments, the carotenoids, about which I have the honour to speak today. In the well-known book by L. S. Palmer on carotenoids which was published in 1922, only six carotenoids are

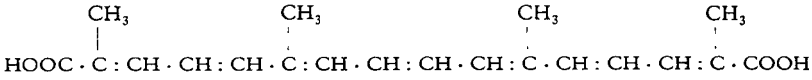
described which had been crystallized and analysed (carotene, lycopene, xanthophyll, lutein, fucoxanthin, and rhodoxanthin). Bixin was also known at that time. Until four years ago the number of these naturally occurring pigments had risen to 15 ; today we know already some 40 natural pigments of this group, the isolation of which has only been made possible by the new methods of separation.

Attention has been paid to the carotenoids in more recent research, not only because of their unique structure, but also because of their close relation to the vitamins, as some carotenoids are provitamins of vitamin A and are transformed into this vitamin in the animal organism. Investigation of the structural constitution of the vitamins started from carotene; when it was possible, in 1930, to establish the correct constitutional formula for b-carotene, a structural formula for any other vitamin or provitamin was not yet known. Shortly afterwards, in 1931, it became also possible to elucidate the constitution of vitamin A itself, and thus to obtain the first insight into the structure of a vitamin. We may perhaps remember that scarcely ten years have elapsed since the time when many research scientists doubted the material specificity of the vitamins, and were of the opinion that a special state of matter, a special colloidal character, was the cause of the peculiar vitamin effects which had been observed.

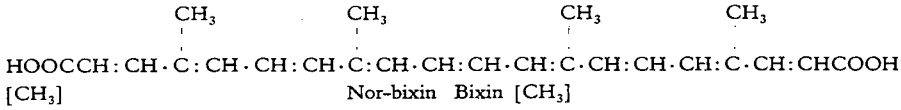
Carotene and its relatives are polyenes; they contain numerous conjugated double bonds in their molecules. We discovered this characteristic structural principle for the first time when investigating the saffron pigment, crocetin. As examples of the molecular structures of such polyenes I give here the formulae of crocetin, bixin (norbixin), the tomato pigment lycopin and fi-carotene (see opposite page).

To the constitutionally clarified carotenoids there also belong a- and y-carotenes, the second yellow Pigment xanthophyll, whose wide distribution in the animal kingdom has been demonstrated by Lomiberg in particular, and also zeaxanthin from maize and many other fruits, the pigment of lobsters and many other crustacea, astacin, the paprika pigment capsanthin, cryptoxanthin, and many others.

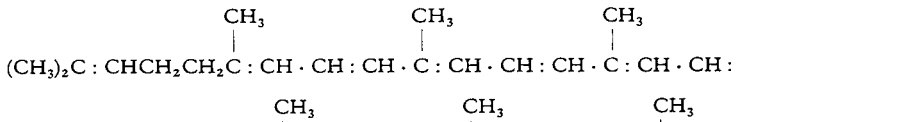
What is striking in the above structural formulae is not only the abundance of conjugated double bonds, unknown previously in any other natural substances, but the fact that the carotenoid molecules are composed of isoprene residues. This applies for all carotenoids which are therefore to be recognized as relatives of the terpenes, camphor, and rubbers. Furthermore, the symmetrical character of the above four formulae is striking; they are composed



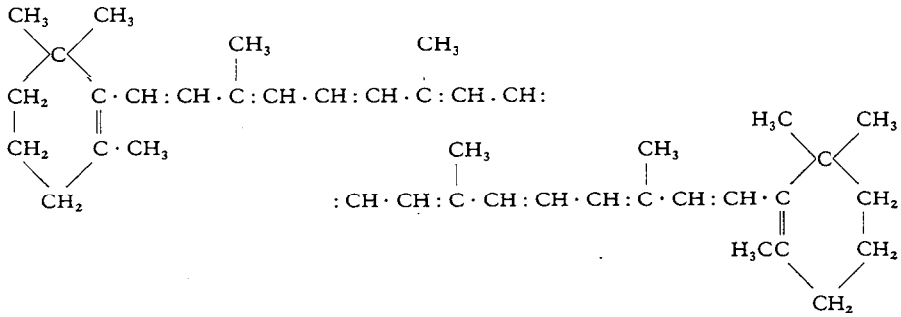
Crocetin



Nor-bixin Bixin



Lycopin



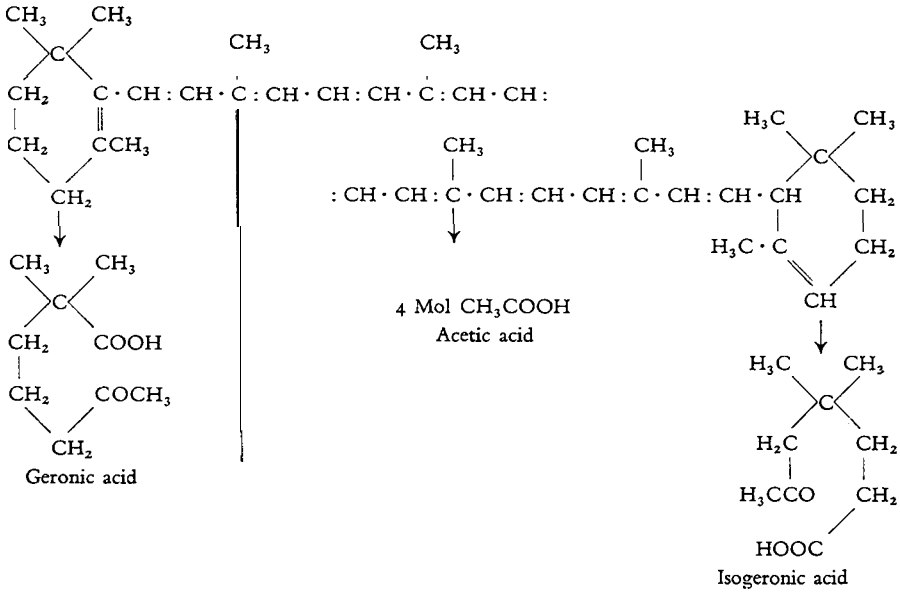
β-Carotene

of two equal halves, and so are centro-symmetrical – both ends of the molecule show the same structure. This structural principle, which we first recognized in the tomato pigment lycopin and then in carotene, later proved to be realized in other branches of terpene chemistry and was applied by various research workers with success in the derivation of structural formulae.

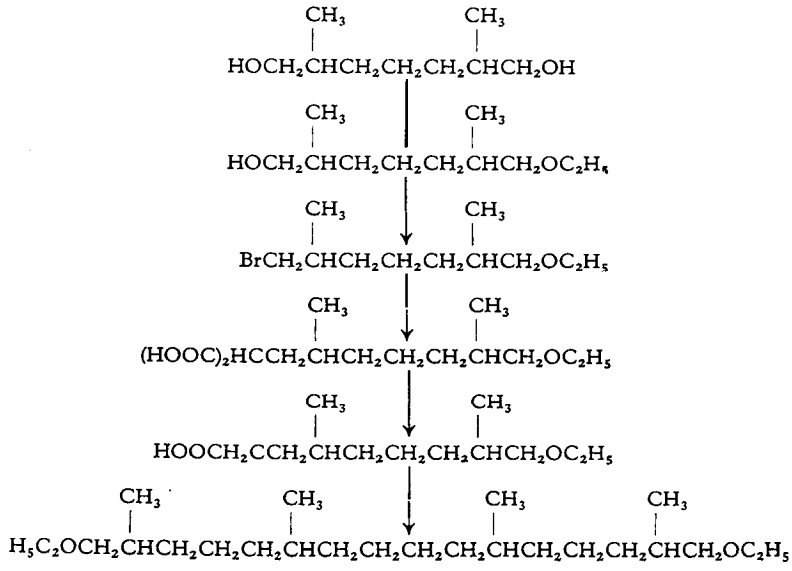
The structural formulae of the above-mentioned four carotenoids have been proved partly by decomposition reactions and partly by synthesis of their perhydroderivatives.

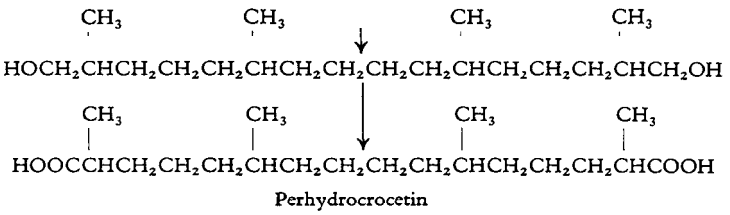
Two examples of this type are shown in the following structural formulae, one showing constitutional determination by decomposition and the other by synthesis.

Oxidative decomposition of a-carotene



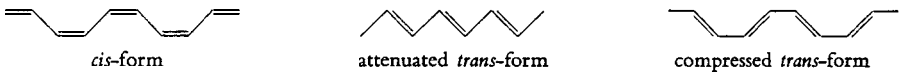
Synthesis of perhydrocrocetin





Perhydrocrocetin

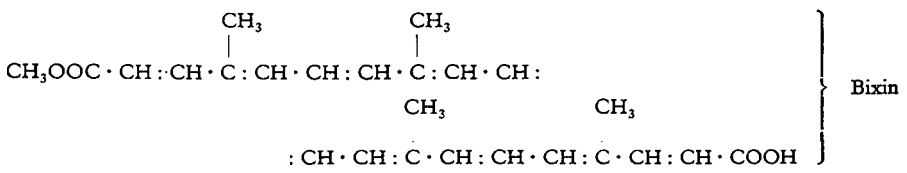
However, the theory now allows us to foresee furthermore for each individual carotenoid extraordinarily numerous stereoisomeric forms, since at each individual double bond the substituents can be arranged either in *cis*-position or *trans*-position, whereby even two modifications of the *trans*-arrangement must be taken into consideration. Diagrammatically the three extreme cases (continuous *cis*-form, continuous attenuated *trans*-form, and continuous compressed *trans*-form) can be represented as follows:

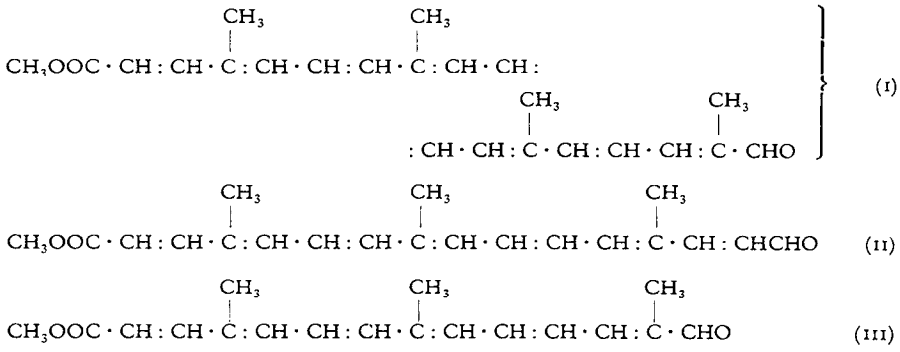


cis-form attenuated *trans*-form compressed *trans*-form

Theoretically a very large number of stereoisomers can be imagined by combining *cis*- and *trans*-arrangements. As in many other cases, however, Nature has made only slight use of these possibilities. Up to the present we know only two carotenoid pigments in which this *cis-trans*-isomerism is realized. These are bixin and crocetin, which occur in both forms as unstable and stable isomers.

Despite the wealth of possibilities of isomerism which are available here, it can be established with great certainty, in the case of the unstable and stable bixin, which double bond is responsible for the occurrence of the two isomers. It is possible to decompose the polyenes by permanganate oxidation in stages to aldehydes in the sense that one double bond after another is oxidized. In this way the aldehydes I to III were obtained from unstable bixin :



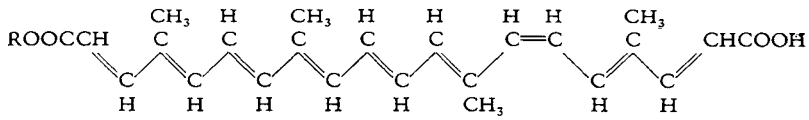


(I)

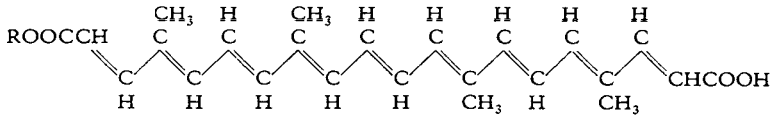
(II)

(III)

In the same way decomposition of stable bixin yields aldehydes of structures I to III. Whereas the compounds III are identical in both cases, the aldehydes I and II obtained from unstable and stable bixin proved to be different; they are *cis-trans*-isomers. From this it can be seen that the stereoisomerism of the two natural bixins is very probably based on the different configuration of the third double bond, counted from the free carboxyl.* If a *trans*-form is assumed for the remaining double bonds (which for various reasons is not improbable, though not proved), then the differences in structure of the two isomeric bixins can be expressed by the following formulae:



Unstable bixin

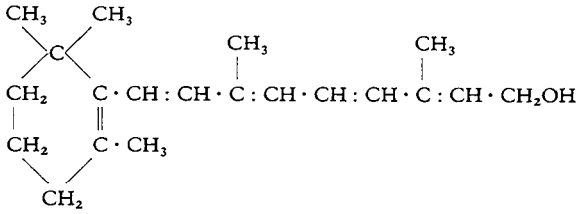


Stable bixin

* An objection to this conclusion could be brought forward, viz. that a labile apo-3-norbixinal methyl ester (Formula III), in contrast to the labile apo-I-norbixinal methyl ester and apo-2-norbixinal methyl ester (Formulae I and II), could rearrange *spontaneously* to a stable form under the conditions of the oxidation.

Even fine stereochemical details of such complex structural molecules are thus experimentally accessible to test and clarification.

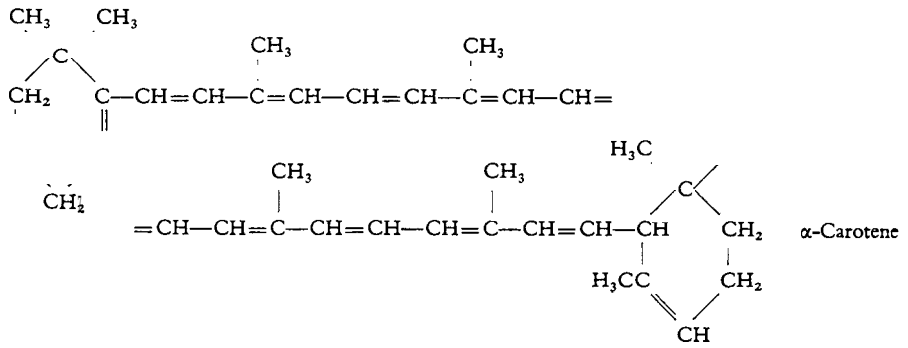
Reference to the close relationship of b-carotene to vitamin A has already been made. H. V. Euler recognized that carotene can replace vitamin A in experiments on animals, and Th. Moore proved that in the animal organism it is transformed into vitamin A. These near relationships became understandable when we succeeded in isolating and constitutionally elucidating vitamin A. The structural formula, which is proved by a synthesis of the perhydro-vitamin A, shows that the compound contains half of the carbon skeleton of b-carotene and from this, by splitting at the central double bond, the result must have been:



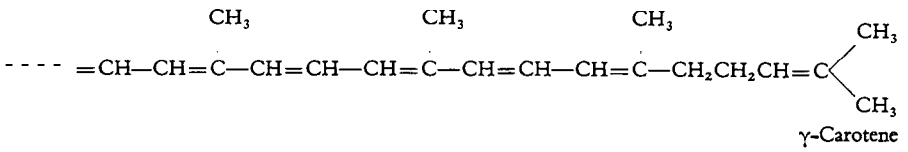
Recently this compound was given the name of axerophtol.

It appeared now to be of interest to establish whether still further carotenoids, besides b-carotene, could be decomposed through the animal organism, presumably through its liver tissue, to axerophtol. Investigation of this problem, which was carried out together with H. v. Euler, led first of all to the recognition that this does not hold true for most other natural carotenoids. Substitution by OH or keto groups, splitting of the carbon ring of b-carotene, etc., leads to a complete disappearance of the vitamin A effect. Besides b-carotene, in whose molecule the unsubstituted carbon skeleton of vitamin A occurs twice, those carotenoids proved still to be effective which contain the carbon skeleton in the unsubstituted form at least once. Pigments of this kind are a-carotene, y-carotene*, cryptoxanthin*, echinenon**, a b-carotene oxide, dihydro-b-carotene, dihydro-a-carotene, diiodo-b-carotene, b-semicarotinone* and also b-apo-2-carotinal and b-apo-4-carotinal. The structural formulae of some compounds of this provitamin group show their close constitutional relationships to vitamin A:

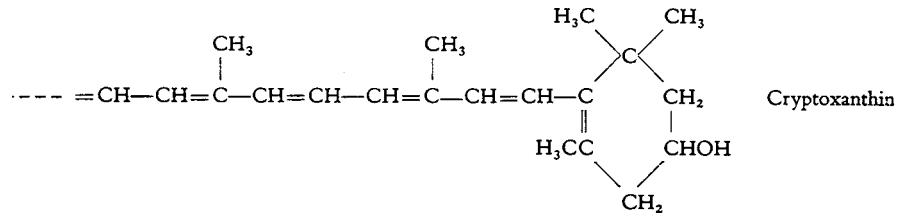
* R. Kuhn *et al.*
** E. Lederer



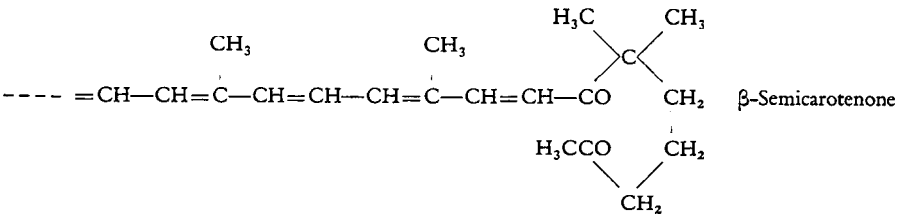
α-Carotene



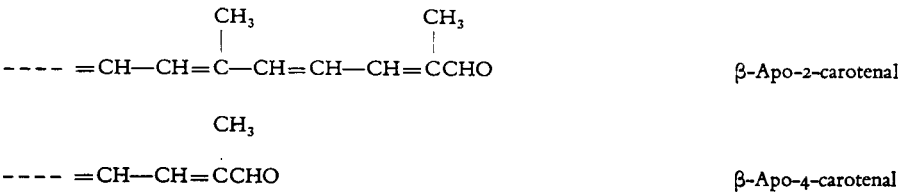
γ-Carotene



Cryptoxanthin



β-Semicarotenone



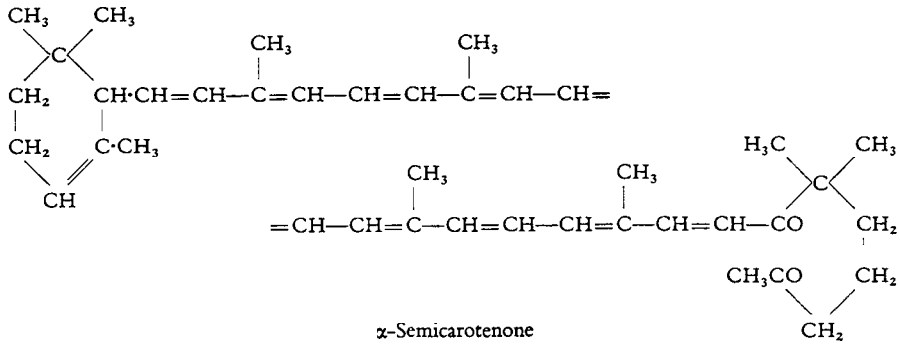
β-Apo-2-carotenal

β-Apo-4-carotenal

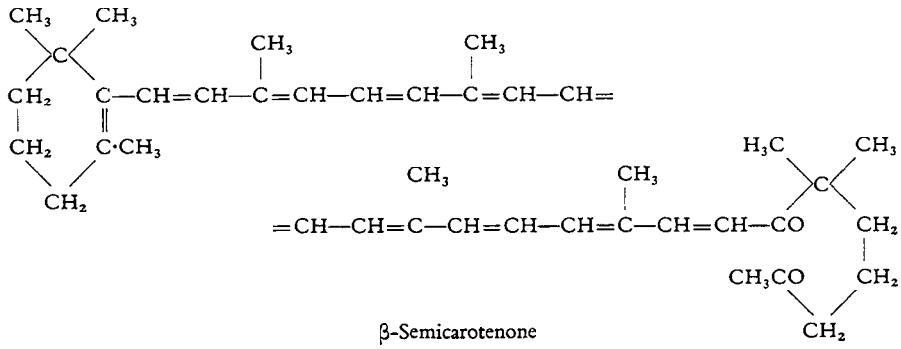
The last three of these provitamins are synthetic products, which have been formed by oxidative splitting at the first, second, or fourth double bond of the carotene molecule.

If a single double bond has changed its location in the carbon skeleton

characteristic for vitamin A, it is sufficient to neutralize its action as provitamin A. In this way cc-semicarotenone, which differs from effective β-semicarotenone by displacement of the double bond in the carbon ring, proved to be quite ineffective:



α-Semicarotenone



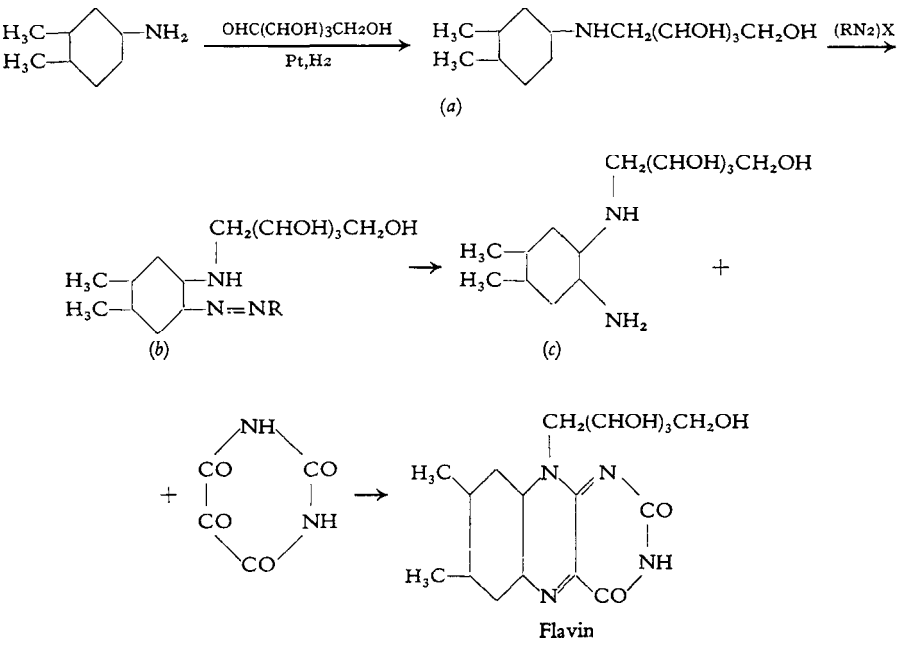
β-Semicarotenone

The action of vitamin A is consequently extraordinarily specific and bound to a quite definite structure. Today it is possible to show the atom arrangement in a polyene characteristic of vitamin A more reliably by experiment on animals than by any chemical decomposition reaction or by spectrum analysis measurements. This high specificity is all the more surprising if it is remembered that with regard to most hormones, e.g. the female sexual hormones and the plant growth hormones, we can hardly speak of a constitutional specificity. Not only do substances which are chemically closely related produce there similar physiological results, but it can often happen that compounds of entirely different constitutions show the same effects as the. natural products.

This strongly pronounced constitutional specificity of vitamin effects may be connected with the results of the most recent investigations, which re-

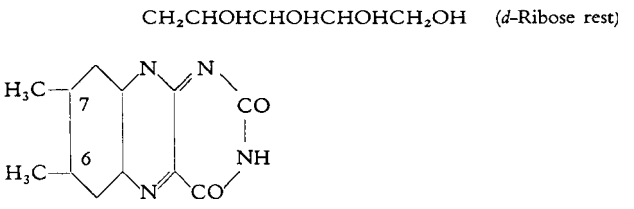
vealed that many vitamins are functional groups of enzymes, the highly specific properties of which have long been known. These close relationships between enzymes and vitamins were first shown in vitamin B_2 or lactoflavin. According to the investigations of Warburg and Theorell the phosphoric acid ester of vitamin B_2 is the functional group of the yellow oxidation enzyme. Today lactoflavin and any other flavin can be obtained synthetically, and hence we were able to investigate at this point to what extent the vitamin effect is bound to a certain constitution.

The process of such a flavin synthesis, one giving the best yields, may be sketched very briefly. Aromatic primary amines are reductively condensed with sugars. These result in compounds of type (a). They can then be coupled with diazonium salts to azo-pigments *(b)*. By reduction, diamino-derivatives of formula (c) are obtained, which combine with alloxan to form the flavin pigment.



By using this and similar methods more than 25 different flavins have been obtained in our laboratory, which differ from one another by the nature of the sugar residues or the substitution in the aromatic ring, amongst them being lactoflavin itself. This experimental material formed the basis

for an assessment of the constitutional specificity of the action of vitamin B_2. As with vitamin A, with lactoflavin the amplitude of variation is extremely narrow. A substitution of the *d*-ribose residue in the lactoflavin



by other sugars practically abolishes the action; only the arabinose compounds still show a weak, stimulating effect. Just as much significance have the two methyl groups in positions 6 and 7. One or the other can indeed still be omitted or substituted by ethyl without the vitamin action being substantially affected. On the other hand, the disappearance of both methyl residues results in complete inactivity. Methyl groups in positions 5 or 8 are unable to restore this again. The action of vitamin B_2 is therefore like that of vitamin A, bound to an exactly circumscribed constitution and configuration.

This specificity makes itself felt not only against higher organisms but also against bacteria. Elvehjem and my former collaborator Strong* were recently able to show that of all the flavins tested, only lactoflavin, 6-methyl-9-(1',*d*-ribityl)-isoalloxazin,7-methyl-9-(I',*d*-ribityl)-isoalloxazin, and 6-methyl-7-ethyl-9-(I',*d*-ribityl)-islloxazin can stimulate the growth of lactic acid bacteria, i.e. the same flavins which are effective in experiments on rats. On the other hand, flavins with other sugar residues, and also the arabinose compounds, had no effect at all.

Up to the present only one natural vitamin A has been found as a decomposition product of carotene - the axerophtol mentioned above. Nevertheless, various observations (Heilbron, Morton et al.) suggest that in certain fish-liver oils, e.g. those of fresh-water fishes, perhaps still other substances occur with a vitamin A action, whose absorption spectra show longer waves than that of axerophtol. There is nothing surprising in this possibility, since we know that a vitamin A effect is peculiar to various synthetic decomposition products of p-carotene *with more than 20 C-atoms*, such as the above-

* The curves on p. 444 were put at my disposal by Dr. Frank M. Strong.

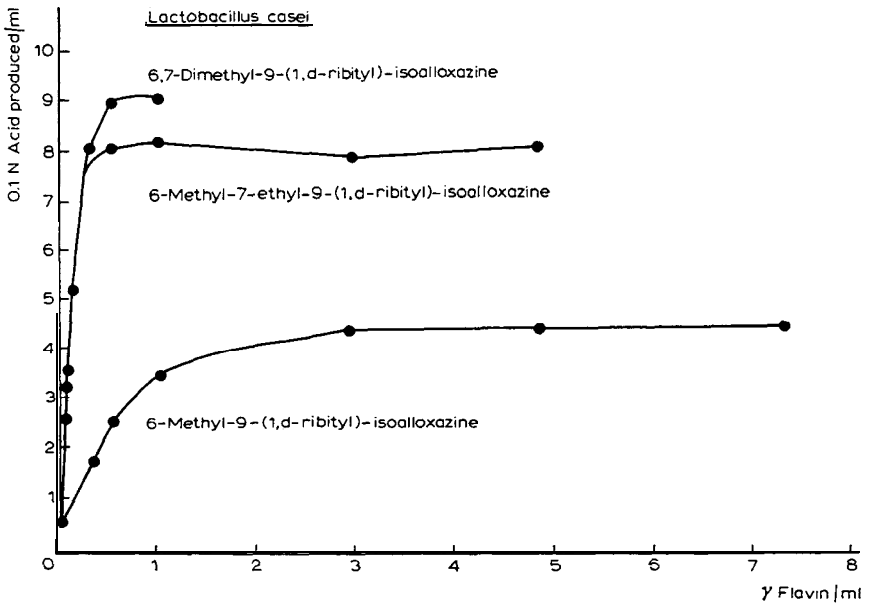

Lactobacillus casei
6,7-Dimethyl-9-(1,d-ribityl)-isoalloxazine
6-Methyl-7-ethyl-9-(1,d-ribityl)-isoalloxazine
6-Methyl-9-(1,d-ribityl)-isoalloxazine
0.1 N Acid produced/ml
γ Flavin/ml

Fig. 1.

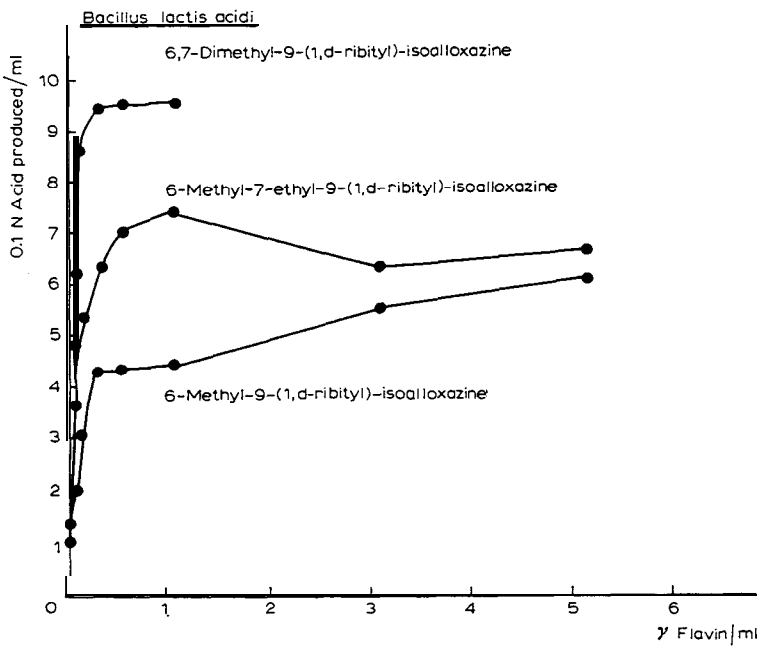

Bacillus lactis acidi
6,7-Dimethyl-9-(1,d-ribityl)-isoalloxazine
6-Methyl-7-ethyl-9-(1,d-ribityl)-isoalloxazine
6-Methyl-9-(1,d-ribityl)-isoalloxazine
0.1 N Acid produced/ml
γ Flavin/ml

Fig. 2.

mentioned apo-carotenals. The thought occurs that Nature too is treading the path which we have been following in the laboratory of late, i.e. in splitting up carotene not only at the central bonds but also at other double bonds, whereby products must result with a longer chromophore system with more double bonds than the ordinary vitamin A possesses. Such substances will be distinguished by absorption spectra of longer wavelengths. Although vitamin A is one of those vitamins whose physiological effects have long been known- Hopkins (1912), Stepp (1911-1912), McCollum, Simmonds, Becker, and Shipley (1922) - even today we are still very much in the dark with regard to the way it intervenes in the cell phenomenon processes. In the cases of vitamin B_2(lactoflavin) and B_1(aneurin), research during the last two years has been more successful in this connection, as the parts played by the first in dehydration reactions and by the second in natural carbohydrate decomposition have been clearly recognized. It is also widely believed today that vitamin A participates in the case of oxidation reduction reactions in the organism. How this intervention happens is, however, still not clearly known.

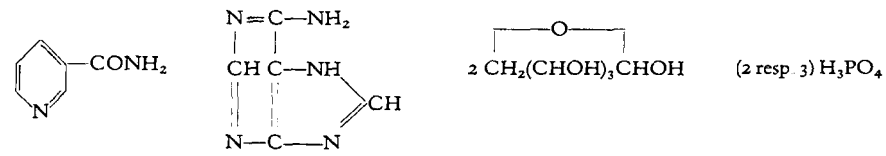
Vitamin A exercises considerable influence on epithelial formation, and many results of an A-avitaminosis are probably the results of the suspension of the effect of the A-factor which promotes the formation of a healthy epithelium. Thus, for instance, A-avitaminosis resulting from injury to the epithelium is connected with lowered resistance to infections; furthermore, in rats it leads to a chronic oestrus (colpokeratose), i.e. the outer layers of the epithelia of the vagina become horny. Modern surgery is making increasing use of the epithelium-promoting effect of vitamin A, by using cod-liver oil preparations, rich in vitamin A, for helping the healing process in wounds; carotene is believed to have a similar favourable effect.

The action of the A-factor on the eyes is remarkably strong; a vitamin deficiency leads to xerophthalmia, the cause of which is sclerotic change, and to night blindness (hemeralopia), a disease which is often encountered, even in these days, among primitive peoples whose nutrition is deficient. It is most remarkable that the eye is one of the organs most rich in vitamins which we know; not only lactoflavin and vitamin C (ascorbic acid), but also vitamin B_2, vitamin K, etc. are present in the eye in considerable quantities. The same applies for the A-factors. Von Euler and Adler have referred to the occurrence of carotenoids in the retina of the eyes of cattle and fish, and G. Wald proved in our laboratory the presence of large quantities of vitamin A (axerophtol) in the retina. According to later in-

vestigations by Wald, vitamin A appears to play an important part in the act of seeing; in the process of exposure to light a pigment, <<retinene>>, combined with a protein and showing a colour reaction with antimony trichloride, is formed from the visual purple. This colour reaction has its absorption maximum at 662-666 mμ. From the retinene, vitamin A is formed (absorption maximum of the antimony trichloride reaction 615 mμ) and finally in the dark the vitamin A is transformed again into visual purple. Eyes kept in the dark contain traces only of vitamin A.

It would seem, therefore, that the chromophore system of the carotenoids is also utilized in the act of seeing, and the animal organism makes varied use of these plants pigments.

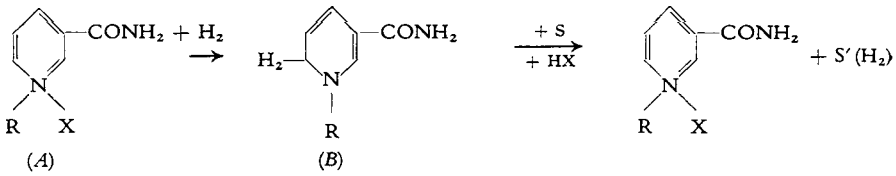
Research into the problems connected with vitamin A took place in a roundabout way; as I have explained before, it began with the chemical treatment and elucidation of the composition of the carotenoids. At that time no one imagined that those investigations would become the foundation for vitamin A research. The same circumstances have applied to various other vitamins. One of the most impressive examples, the last to become known, is the story of nicotinic acid amide. This was recognized by Warburg as a constituent of a coenzyme, codehydrase II, and by von Euler as the building material of the cozymase (codehydrase I), in which it occurs together with adenine, pentose, and phosphoric acid:



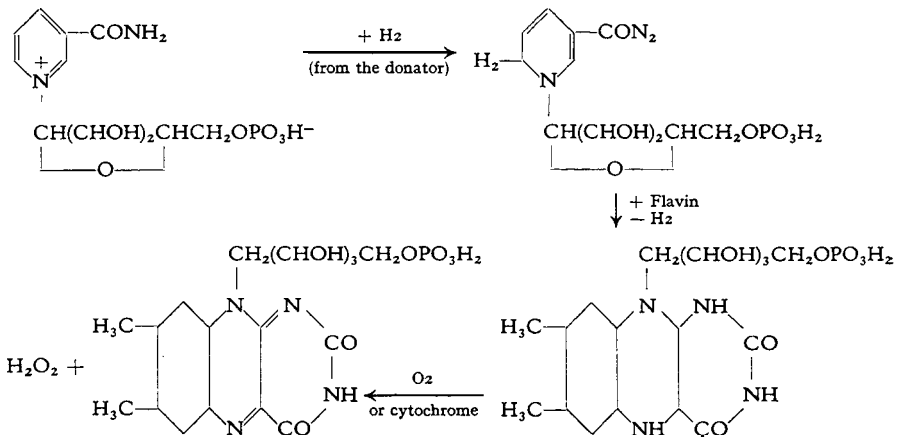
Nicotinic acid amide Adenine Pentose Phosphoric acid

Then, together with O. Warburg, we were jointly able to show that the hydrogen-transferring role, i.e. the central significance, devolves precisely upon this constituent of the two codehydrases in the oxidation-reduction processes.

A hydrogen transference was found to be possible only through such nicotinic acid amide derivatives in which the pyridine nitrogen has a quarternary character. Such compounds (Formula A) are transformed by assimilating the hydrogen split from the substrate into ortho-dihydro derivatives (Formula B), which then pass on the hydrogen to other compounds (S):



In the two codehydrases it is the sugar residue, the pentose residue, which stands in quatemary bond at the pyridine nitrogen of the nicotinic acidamide; the dehydration of the resultant dihydronicotinic acid compound is effected through the chromophore system of the yellow enzyme, i.e. through lactoflavin (vitamin B$_2$). The entire dehydration process is represented by the following structural formulae :



As a result of these investigations nicotinic acid amide suddenly became of general interest. It was tried as a remedy in the most varied kinds of diseases, and in doing this Elvehjem and his co-workers discovered that it is the cure for the <<black tongue, disease in dogs, on which experiments had been carried out for decades. We are therefore dealing in this instance with a newly discovered supplementary factor in nutrition, a new vitamin.

It has often been said that it is only the first discovery which is difficult and that the ensuing discoveries are usually only the continuations of the first. Perhaps vitamins and carotenoids offer examples of this.

In a lecture which I gave in 1932 I remarked: <<The chemistry of the vitamins has made great progress in the last few years; it has overtaken the

chemistry of the hormones and left that of the enzymes far behind. Soon we shall be so far advanced that all kinds of positive statements can be made about the chemical nature of the different vitamins. Then the chemist will be able to hand back the vitamin problem to the physiologist, so that the latter may establish in what way these substances develop their effect in the organism. Once physiology has solved this problem, it will have become enriched by one of its greatest findings.>>

This prophecy has been fulfilled to a large extent within the short space of five years. The chemical side of the vitamin problem is solved in its essential points; relationships to enzymes were found. It is the task of physiology today to explain the intervention of these agents in the cell processes. As this is, however, a matter of chemical processes, these will in the end probably have to be elucidated by the chemist once again.

Biography

Paul Karrer was born in Moscow on April 21, 1889. His parents, Paul Karrer and Julie Lerch, were Swiss nationals and in 1892 the family returned to Switzerland where he received his early education at Wildegg and at the grammar school in Lenzburg, Aarau, where he matriculated in 1908 He studied chemistry at Zurich University under Professor Alfred Werner and after gaining his Ph.D. in 1911, he spent a further year as assistant in the Chemical Institute. In 1912 he took a post as chemist with Paul Ehrlich at the Georg Speyer Haus, Frankfurt-am-Main; he left Frankfurt six years later on his election as reader at Zurich University. In 1919 he became Professor of Chemistry and Director of the Chemical Institute.

His early researches involved the preparation and investigation into the properties of complex metal compounds but his most important work has concerned plant pigments, particularly the yellow carotenoids. He was responsible for elucidating the chemical structure of the carotenoids and he also showed that some of these substances are transformed in the animal body into vitamin A. His work in this field led, in 1930, to the establishment of the correct constitutional formula for b-carotene, the chief precursor of vitamin A; this, the first time that the structure of a vitamin or provitamin had been established, in turn led to the clarification of the structure of vitamin A itself. Later, he confirmed the structure ascribed to ascorbic acid (vitamin C) by Albert von Szent-Györgyi and he extended his researches into the vitamin B_2 and E fields. His important contributions to the chemistry of the flavins led to identification of lactoflavin as part of the complex originally thought to be vitamin B_2.

Professor Karrer has published over 1,000 scientific papers in the various fields of organic chemistry, especially concerning vitamins A, B_2, C, E and K, co-enzymes, carotenoids and other plant pigments, curare and other alkaloids, amino acids, carbohydrates and organo-arsenic compounds. His Lehr*buch der Organischen Chemie* (1930) has passed through 13 editions and has been translated in full into English, Italian, Spanish, French, Polish and Japanese. His monograph on carotenoids (1948) has also been translated into English.

Karrer was President of the 14th International Congress on Pure and Applied Chemistry (Zurich, 1955). He has received honorary doctorate degrees from universities in Europe and America; they include Dr.med. Basle, Breslau, Lausanne and Zurich; Ph.D.Lyons, Paris, Sofia, London, Turin, Brussels and Rio de Janeiro; and Dr. Pharm. Madrid and Strasbourg. He has been awarded the Marcel Benoist Prize and the Cannizzaro Prize and he is a full member or honorary, corresponding or associate member of numerous chemical and biochemical societies throughout the world. These include the Académie des Sciences (Paris); the Royal Society (London) ; National Academy of Science (Washington); Royal Academy of Sciences (Stockholm) ; the National Academy (Rome); Royal Academy of Belgium ; the Indian Academy of Science ; the Royal Netherlands Academy of Sciences, and the Chemical Societies of Britain, France, Germany, Belgium, India and Austria.

Karrer married Helena Froelich in 1914. They have two sons.