THE CHEMISTRY OF VITAMINS A AND C[1]

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Absence or insufficiency of vitamins in the daily food ration will cause certain well-defined symptoms of diseases. To these biological effects we really owe the discovery of the vitamins; they were also absolutely essential for their purification and final isolation. Eight or nine vitamins are known today, and only four of these have been prepared in a pure, or at least nearly pure, state. These are vitamin A, the fat-soluble factor of growth; B_1, the antineuritic vitamin; C, the antiscorbutic vitamin; and D, the antirachitic vitamin. Only for vitamins A and C can structural formulas be advanced. These two have also been a principal subject of our own investigations, and therefore, I shall devote myself mainly to them in this lecture.

I begin with the antiscorbutic factor, vitamin C. Zilva several years ago showed that highly purified vitamin C preparations were free from nitrogen, and that the antiscorbutic factor must have a molecular weight like a hexose. At about the same time C. G. King greatly improved the methods for concentrating vitamin C in lemon juice, and Tillmans made the important observation that the reducing power of many plant juices towards iodine and other weak reductive agents, like dichloroindophenol, in acid solution, is in proportion to the antiscorbutic efficiency of these plant juices. From this parallelism he concluded that vitamin C itself must be a powerfully reducing substance.

About five years ago Szent-Györgyi isolated from the cortex of the adrenal gland and later on also from plants, a well crystallizing substance, having the empirical formula $C_6H_8O_6$. In acid solution it showed strong reducing power against iodine and similar

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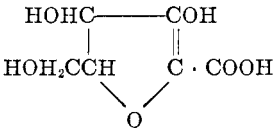
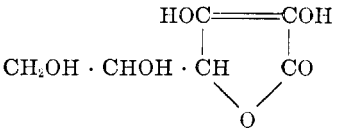
oxidative substances. In 1932 C. G. King isolated the identical substance from lemon juice and discovered that it cured animals suffering from scurvy. Simultaneously, after Tillmans' investigations in regard to the reducing properties of the antiscorbutic factor had become widely known, Szent-Györgyi tested his substance for antiscorbutic properties and found that it had a curative effect on guinea pigs in doses of 0.5–1 mg. per day. To this substance the name ascorbic acid was afterwards given. Zilva, Dalmer and Moll, Tillmans, von Euler, and others confirmed its efficiency in healing scurvy.

We fractionated by recrystallization 25 g. of ascorbic acid, until the last fraction, the least soluble one, amounted to about 1 g. We tested every fraction for its reducing power against iodine. No differences could be detected. According to von Euler and Demole the antiscorbutic efficiency of all fractions was also identical. There is therefore hardly any doubt about the homogeneity of this substance.

The constitution of ascorbic acid has been the subject of a number of investigations. I mention Szent-Györgyi with his coworker Vargha, Haworth, Hirst, Smith and Reynolds, Micheel, and finally also our own institute in Zürich. It is certain that ascorbic acid is a monobasic acid, with four hydroxyls in all. Two of them are enolic and account for the acid character, and two are alcoholic hydroxyls, of which one is primary, giving a triphenylmethyl ether. The other alcoholic hydroxyl must be in either the 2- or the 3-position to the first one, as ascorbic acid forms with acetone a well crystallizing acetone compound; this reaction is characteristic for 1,2- or 1,3-glycols. Micheel oxidized the dinitrobenzoic acid ester of the enolic methyl ether of ascorbic acid and found among the products of degradation *l*-threonic acid and oxalic acid. This result would give to either one of the two following formulas a high degree of probability.

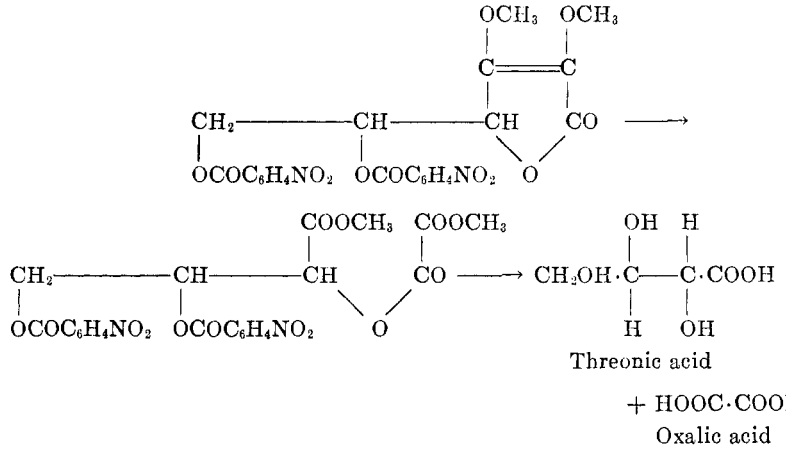


I

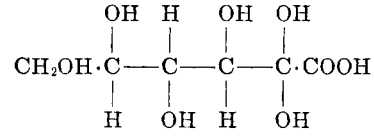


II

According to I, ascorbic acid would be a furan derivative, according to II, a γ-lactone (Hirst, v. Euler). If we assume the lactone structure the degradation would proceed as follows:



At present the lactone structure seems to be the more likely one. Dimethylascorbic acid yields with alcoholic ammonia a derivative containing nitrogen, and the two methoxyl groups are still intact. It can be regarded as an amide resulting from the γ-lactone through an opening of the lactone ring. The formation of idonic acid

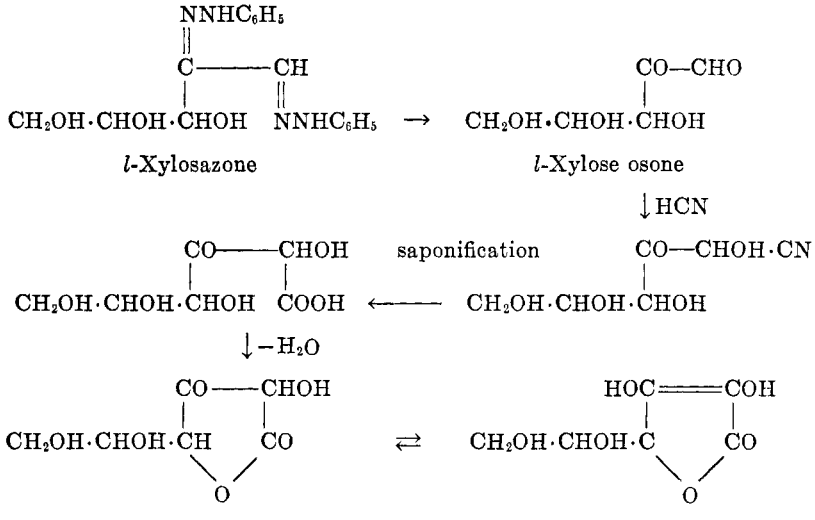


by catalytical reduction of ascorbic acid also speaks in favor of this lactone structure.

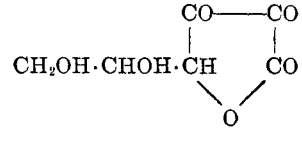
That the lactone structure is the correct one, we could recently prove in the following manner: Dimethylascorbic acid reacts with Grignard reagent without splitting off a methoxyl group. This is only possible if ascorbic acid is a lactone, as a methyl ester would react by removing of one mole of methyl alcohol.

Reichstein in Zürich has succeeded in preparing ascorbic acid by synthesis. The synthesis would be in good agreement with the lactone structure, II, or at least one very closely related to it. It would however not exclude the furan structure I. Starting from

l-xylose, Reichstein prepared first the osazone and from this the osone. Treatment of the osone with hydrocyanic acid gave a nitrile, and the saponification of the latter yielded ascorbic acid. The yield was about 20 per cent figured on the osone. Chemical and physical properties are absolutely identical with those of ascorbic acid. The vitamin C efficiency is now being tested.



The most characteristic chemical property of vitamin C is its extraordinary power of reduction. In acid solution it uses an amount of oxygen corresponding to the oxidation of two hydrogen atoms. The product of this oxidation, the dehydro-ascorbic acid, can easily be reduced again to its mother substance, for instance, by means of sulphuretted hydrogen. The dehydro acid was isolated by us in pure, but amorphous form. The analysis shows that it has two hydrogen atoms less than ascorbic acid, and the determination of the free hydroxyls, according to Zerewitinoff, gives only two hydroxyls. This result can only be explained by assuming that the oxidation has removed the hydrogen from two of the hydroxyl groups, most likely forming a diketone of the constitution:



Dehydroascorbic acid has not lost its antiscorbutic efficiency; in fact, it shows about the same activity as ascorbic acid itself.

For guinea pigs afflicted with scurvy, the healing dose of vitamin C is at least 0.5 mg. per day. This dose is about 100,000 times as large as the curative dose of vitamin D, and 10,000 times the amount needed of vitamin A. There is of course the possibility that ascorbic acid is not the vitamin itself, but that it acts as provitamin, from which the organism only creates the C-factor. However there is really at present no basis for this hypothesis.

Pure ascorbic acid has now become an easily accessible vitamin, obtainable in any desired amount. This will undoubtedly give rise to important researches, physiological as well as biochemical. I am quite sure that very interesting new functions of this vitamin will yet be discovered. We have already observed that ascorbic acid acts as activator of the catheptic proteoses. Catheptic proteolytic ferments, which have lost their natural activators and have become inactive, can be reactivated with ascorbic acid. In this respect vitamin C resembles cystein and glutathione, two substances which, according to Waldschmidt-Leitz, also undergo reversible oxidation. This coferment effect depends perhaps on the oxido-reductive potential.

I now proceed to my next subject, vitamin A. This is one of several vitamins necessary for the proper growth of the animal. It therefore is called the fat-soluble growth factor. However, deficiency of vitamin A not only produces loss in weight, but also hemeralopia, xerophthalmia, degeneration of nerve fibers, skin diseases, and affections of the mucous membrane, etc.

The history of vitamin A is still young, but has nevertheless been full of interesting episodes. Osborne and Mendel, McCollum and Davis were the first who ascertained the presence of factor A in cod-liver oil and butter. Hopkins found that cod-liver oil loses its curative qualities if air is bubbled through it. Then came Steenbock and his school with their thorough and important investigations. They examined numerous plants and their extracts, also food stuffs, as to their vitamin A content. They brought out the interesting fact that only such vegetable and similar products had a vitamin A effect, as were rich in certain yellow pigments, the so-called carotenoids. From this they concluded that vitamin A must stand in some relation to these pig-

ments. Steenbock tested carotene for vitamin A effect, and claimed that it had curative properties. Drummond repeated these tests, and found that it was ineffective. He drew the conclusion that Steenbock's positive results must have been due to some foreign substance accompanying the carotene, and for years this remained the generally accepted opinion.

In 1927 von Euler was able to harmonize these contradictory statements. Drummond had used a vitamin A-free diet plus carotene. von Euler's investigations established the important fact that the above ration had indeed no vitamin A curative effect. The cause of this, however, was not inefficiency of carotene, but the fact that this ration was not only devoid of vitamin A, but also of vitamin D. Vitamin A however can only exert its curative powers in presence of vitamin D and the other vitamins. von Euler therefore added carotene and vitamin D to the ration used by Drummond and now the curative effects were the same as of good cod-liver oil, which as you know is rich in both of these vitamins.

These new facts, however, were not generally accepted. It was argued that cod-liver oil, a rich source of vitamin A, does not contain any carotene, but some other substance with an absorption spectrum totally different from that of carotene. Th. Moore finally solved the riddle. The liver is the storage house for vitamin A. Moore found that the vitamin A content of the liver decreases rapidly and finally disappears completely, if animals are kept on a vitamin A-free diet. After an addition of carotene to the diet, the vitamin A content of the liver increases again rapidly. The explanation is, that carotene is a provitamin of the A factor which means that it is transformed by the organism into the vitamin.

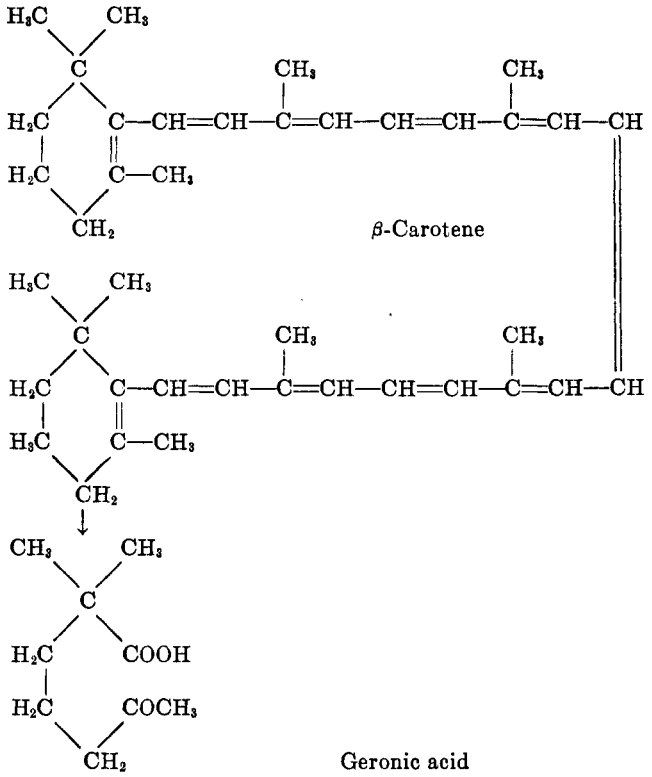
von Euler and our own investigations have shown that of all naturally occurring carotenoids, only the carotenes have this provitamin A effect. Vegetable carotene is hardly ever homogeneous. It is a mixture of two or more isomeric forms, known as α-, β-, γ-, and possibly even still another one. The γ-carotene of Kuhn is present only in traces; β-carotene is always the main component and can perhaps occur without any of the others. The amount of the α-carotene varies greatly, from traces to 20 or more

per cent. The separation of these various isomeric carotenes from each other was a tedious and not very satisfactory process. Fractionation by crystallization, adsorption on specially prepared aluminum hydroxide or Fuller's earth, give a certain degree of purification and enrichment of one of the components. To get a complete separation, the process must however be repeated over and over again. In search of better adsorbents we recently found that calcium hydroxide or calcium monoxide are greatly superior. The process is carried out like a chromatographic analysis. One adsorption suffices to bring about a clean separation of α-carotene from β-carotene, and α-carotene is now a readily accessible substance. α-, β-, and γ-carotene have slightly different melting points and solubilities. Their absorption spectra also show different lines. Only α-carotene is optically active. (See table 1).

TABLE 1

	MELTING POINT	MAXIMA OF ABSORPTION (CS_2)	$[\alpha]_{544}$
	degrees C.	$\mu\mu$	
α-Carotene, $C_{40}H_{56}$	183	509, 477, 448	+323°
β-Carotene, $C_{40}H_{56}$	183	520, 485, 450	Inactive
γ-Carotene, $C_{40}H_{56}$	174	533, 496, 463	Inactive

The determination of the constitution of these carotenes and especially of the most important one, β-carotene, has been the subject of long investigations at our Zürich institute. Today the structural formula has been definitely proven. β-carotene, $C_{40}H_{56}$, is an unsaturated hydrocarbon, containing eleven double linkages. Through catalytic reduction it takes up eleven moles of hydrogen. Degradation by oxidation has been particularly useful for elucidating the chemical structure. Ozonization gave geronic acid, permitting the conclusion that the carotene molecule must contain carbon rings like β-ionone. Comparing the yields of geronic acid obtained by ozonization of β-ionone and β-carotene, we came to the conclusion that the carotene molecule must contain two β-ionone rings as expressed by the following formula:



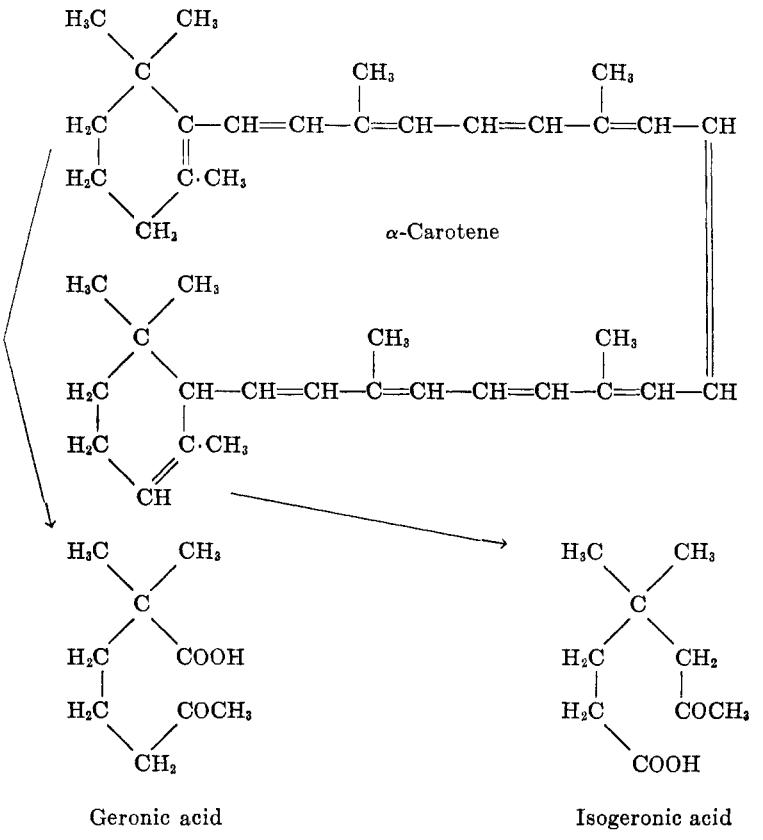
β-Carotene

Geronic acid

Degradation of β-carotene with potassium permanganate gives four moles of acetic acid; with chromic acid six moles. This proves six methyl groups in the molecule. Four of these, those which give acetic acid already with permanganate, belong to the aliphatic chain.

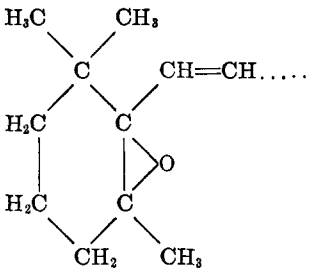
Time is too short to give all the reasons for this formula for β-carotene. It suffices to say, that it is so far in excellent agreement with all experimental data.

α- and β-carotene are isomeric, and their isomerism is due to the different position of the double linkages. Ozonization of α-carotene yields geronic acid, and in contrast to β-carotene also isogeronic acid. The only formula which fully explains these results is the following:



The asymmetric C atom in α-carotene accounts for the optical activity of this substance.

α-, β- and γ-carotene are not the only provitamins of the A factor. They are the only ones, as far as we know, occurring in nature. Four carotene derivatives however have been prepared, which can act as provitamins. The first of these is carotene iodide, a well crystallizing substance. Another one is the dihydrocarotene, which is a product of partial catalytic reduction and probably not a homogeneous substance. The third is a monohydroxy carotene, and the last an oxide of carotene, whose oxygen atom is most likely in the form of an inner ether.



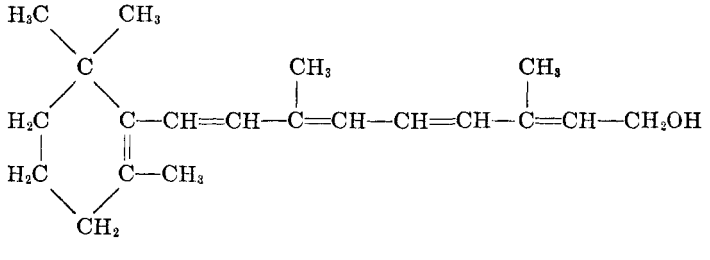
In all four of these derivatives, at least one of the original two β-ionone rings is still unchanged. If both of these rings are altered, as is the case in the xanthophylls or phytoxanthins, through substitution of hydrogen atoms by hydroxyl groups, the ability to act as provitamins disappears completely. The presence of at least one β-ionone ring is therefore absolutely essential for the vitamin A effect. This fact is no longer surprising, since we know the close relationship between β-carotene and vitamin A.

Liver oils from various animal sources differ greatly in their vitamin A content. Not only does the amount vary between the different species, but it is also greatly influenced by their food. If the food is rich in carotene, the vitamin A, which is stored in the liver, increases enormously and far above the normal amount. Fish-liver oils are much richer in vitamin A in summer than in winter: for instance, halibut liver oil contains twenty times more in summer. According to von Euler's and our own investigations the liver oils from Hippoglossus hippoglossus, Scombresox saurus, Rhombus maximus and of the Japanese fish, Stereolepis ishinagi, contain from 200 to 2000 times as much vitamin A as common cod-liver oil. The discovery of this fact was of great importance. Without it we would hardly have been able to carry on our investigations.

Fractionation, the removal of foreign substances by freezing out at very low temperatures, and methods of adsorption led finally to vitamin A preparations with constant analytical data. These preparations could at least be regarded as highly concentrated forms of vitamin A.

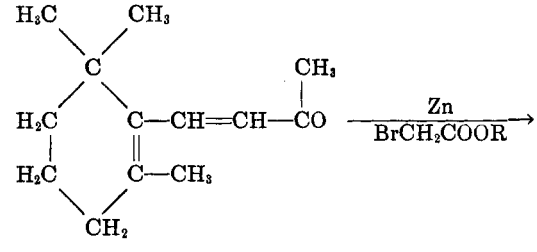
This period of my work will always remain fixed in my memory as one of the most fascinating of my laboratory experiences. Up to the time when we started using those liver oils, very rich in

vitamin A, we had of course experimented only with the unsaponifiable residue from cod-liver oil. Tedious processes of purification and concentration gave us products with as high as 400 C.L.O. units, the usual standard for measuring the vitamin A content. You can well imagine what it meant to us, when we found oils where this unsaponifiable residue, without any further purification, showed already C.L.O. units as high as 200 to 800. Methyl alcoholic solutions of these crude residues were purified by freezing out the sterines and other foreign substances at low temperatures. The C.L.O. unit rose promptly to 5000. A repetition of this process at 70°C. below freezing gave a product with 8000 C.L.O. units. Fractional adsorption on fibrous aluminum hydroxide gave a further rise to 9000, and a second adsorption finally even to about 10,000 C.L.O. units. Then it stopped, and renewed adsorptions on aluminum hydroxide did not give any further improvement. Quite recently we found in calcium hydroxide a much better and especially more selective adsorbent. Through adsorption on calcium hydroxide a small amount of a foreign substance with a distinctly different absorption spectrum can be removed. The analytical data of the main fraction, however, remain unchanged. The final product is a very viscous light yellow oil. It can be distilled without decomposition under very much reduced pressure, boiling at a temperature of 137–138°C. Our investigations resulted in the following formula for vitamin A.

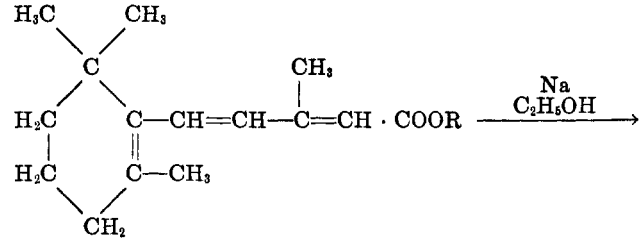


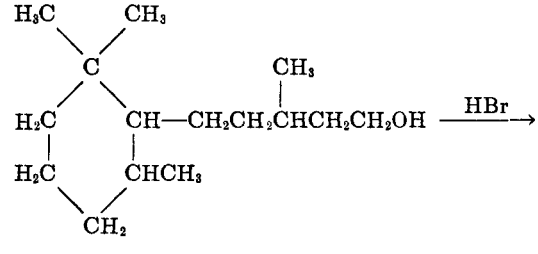
The degradation of vitamin A by oxidation is most illuminating and of great importance. Just like β-carotene, it gives geronic acid and three moles of acetic acid. Esterification establishes one alcoholic hydroxyl group. Catalytic reduction indicates five double linkages. Quite recently we synthesized the perhydrovitamin A and now the structural formula has become a certainty.

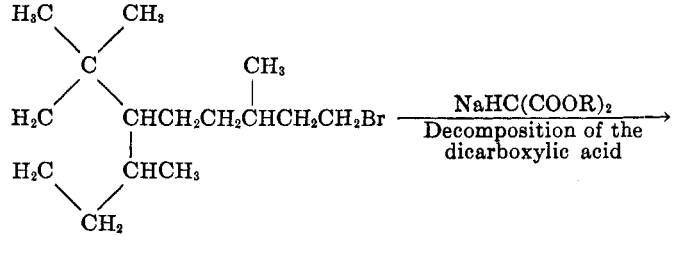
We started from β-ionone and, by way of many intermediates, built up the perhydro-vitamin A. This, however, like the natural perhydro-vitamin A, is an oil. To make absolutely sure we therefore had to proceed one step further, and synthesize the higher ketone K. We did this with the perhydro-vitamin A, obtained by catalytical reduction of the natural vitamin, as well as with our synthetic product. Both ketones gave well-crystallized semicarbazones, identical in every respect.

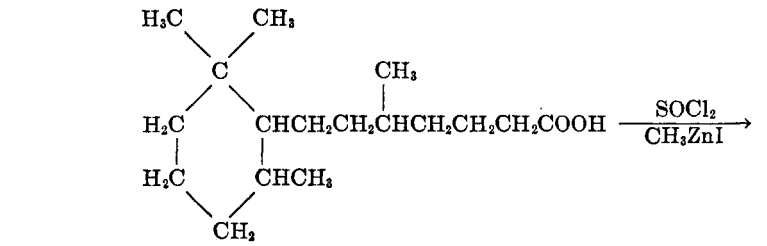


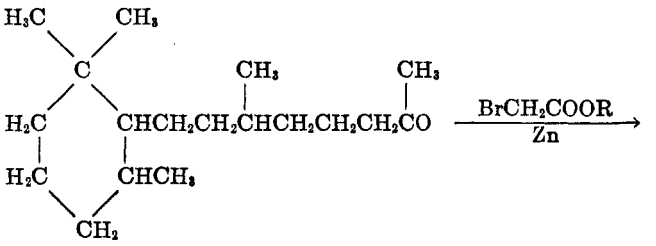
β-Ionone

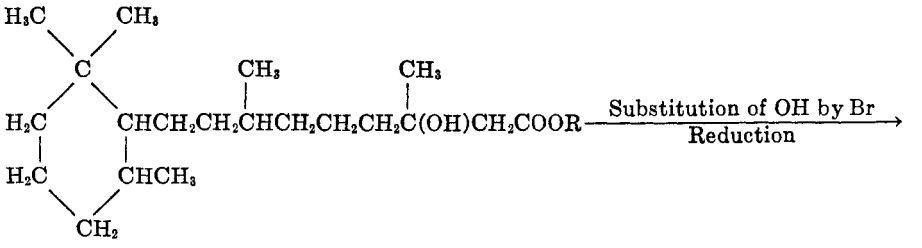


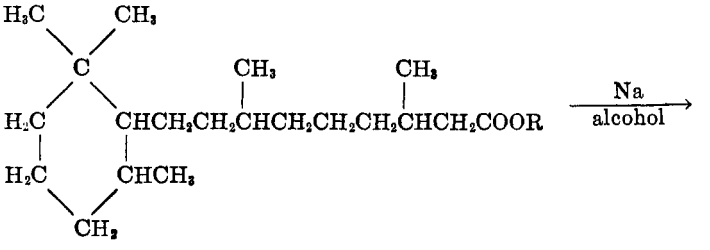


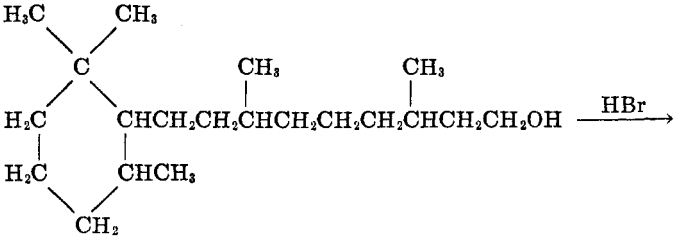




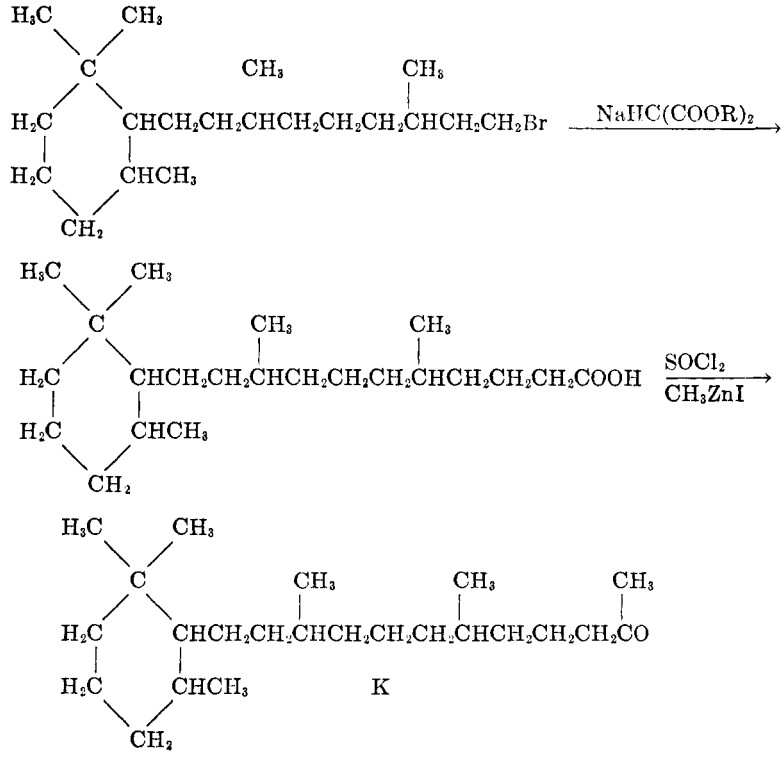








Perhydro-vitamin A



A comparison of the formulas of β-carotene and vitamin A clearly reveals their structural and genetical relationship. The β-carotene molecule is split in half and one alcoholic hydroxyl appears at the end of the open chain.

Scientists of many nations have harmoniously worked together in the new field of vitamins. Americans and American institutions, however, can claim the honor of having been the real pioneers. They were the first to tackle this most difficult and unpromising problem. They used strictly chemical and physiological methods for the isolation and identification of these, at that time nearly mythical, substances. To them science owes a great debt of thanks. For me it has therefore been a special pleasure and a still greater honor that I should have been permitted to deliver this lecture before your Society. I want to express to you my sincerest thanks and appreciation.