Recent Developments in the Vitamin A Field.

THE PEDLER LECTURE DELIVERED ON DECEMBER 4TH, 1947.

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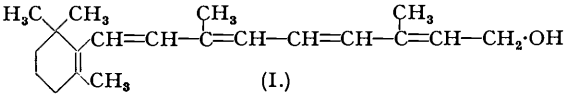
ALTHOUGH the primary object of this lecture is to place before you some of the recent developments in the chemistry of vitamin A which have led to the ultimate goal of synthesis, in order that my narrative may be complete I would like briefly to recall some of the more salient points in its early chapters.

It is difficult precisely to state the actual year in which the vitamin was recognised as a specific substance, but the discovery is generally ascribed to McCallum and Davis who found that whereas growth ceased prematurely with young rats fed on synthetic diets in which fat was supplied as lard, it was immediately resumed if either butter fat or an ether extract of egg yolk was added to the diet (*J. Biol. Chem.*, 1913, **15**, 167; cf. also Osborne and Mendel, *ibid.*, p. 311). In 1922, clear evidence was adduced that animal fats and fish-liver oils contain two distinct vitamins, the one now known as vitamin A, which was found to be directly associated with growth, antixerophthalmic activity, and night blindness, and the other, vitamin D, the antirachitic factor (McCallum *et al.*, *ibid.*, 1922, **53**, 293). The next few years saw attention mainly directed to the working out of satisfactory methods of the detection and estimation of the vitamin.

First and foremost came, of course, the biological assay, the final arbiter of physiological potency. This process is unsatisfactory for routine assay purposes, being both time-consuming and laborious; it has now been largely superseded by physical methods. Vitamin A concentrates give a blue colouration with chloroformic antimony trichloride (Carr and Price, *Biochem. J.*, 1926, **20**, 497) the intensity of which depends on the vitamin A content. This may be estimated either colorimetrically by comparison with a standard vitamin A concentrate, or more specifically in terms of the intensity of light absorption at the exhibited maximum, 620 mμ (Gillam and Morton, *ibid.*, 1931, **25**, 1346). The most reliable method of vitamin essay is based on the observation of Morton and Heilbron (*ibid.*, 1928, **22**, 987) that vitamin A exhibits characteristic maximal absorption at 328 mμ, the intensity of light absorption at this point giving a direct measure of the vitamin A content, generally expressed in terms of the extinction coefficient, $E^{1\%}_{1\,cm.}$. An excellent critical survey of the methods of assay of vitamin A and of the need for a satisfactory standardisation of the " International Unit " has been recently made by Gridgeman (*Chem. and Ind.*, 1947, **38**, 574).

The discovery by Poulson in 1929 (*Strahlentherapie*, 1929, **34**, 648) that halibut-liver oil constituted a rich source of the vitamin pointed the way to its isolation in the pure state. Viscous golden-yellow concentrates of up to 70% purity were obtained from the crude oil by application of the methods of chromatography and of molecular distillation (Karrer, Morf, and Schopp, *Helv. Chim. Acta*, 1931, **14**, 1036, 1431; Heilbron, Heslop, Morton, Webster, Rea, and Drummond, *Biochem. J.*, 1932, **26**, 1178). It was not until 1942, however, that Baxter and Robeson succeeded in obtaining pure vitamin A in the form of pale yellow prisms, m. p. 63—64° (*J. Amer. Chem. Soc.*, 1942, **64**, 2411).

Although the isolation of the crystalline vitamin is of such a recent date, its constitution as the polyene alcohol (I) was established as early as 1931 by oxidative degradation experiments (Karrer, Morf, and Schopp, *loc. cit.*). Confirmatory evidence was provided by Heilbron, Morton, and Webster (*Biochem. J.*, 1932, **26**, 1194) who dehydrogenated vitamin A with selenium and isolated 1 : 6-dimethylnaphthalene, thus determining the relative positions of the *gem*-dimethyl group and the first side-chain methyl group. Synthetic evidence for the postulated carbon skeleton was provided by Karrer and Morf (*Helv. Chim. Acta*, 1933, **16**, 625) who established the identity of perhydrovitamin A, the fully hydrogenated compound, with a compound synthesised from β-ionone.



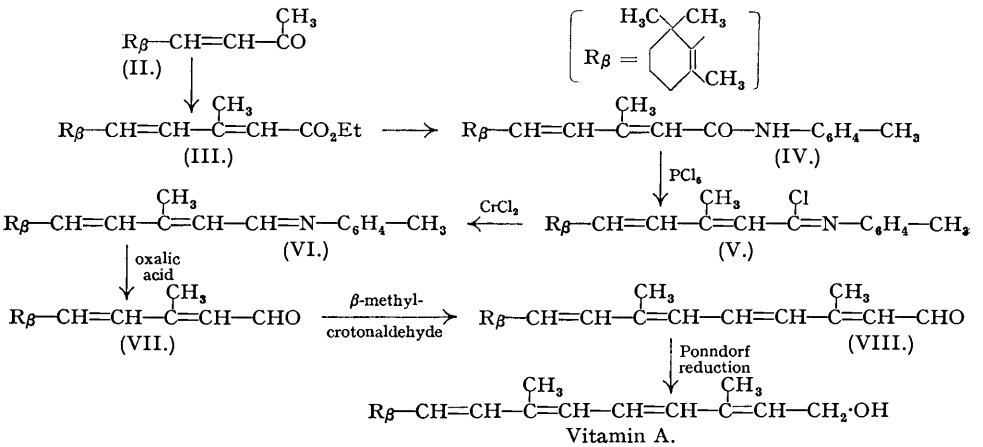
(I.)

Following upon the elucidation of the structure of vitamin A, its synthesis provided the next objective, and this problem has been actively pursued throughout the past decade by workers in many countries.

The most obvious starting point is β-ionone (II), but unfortunately, apart from its normal

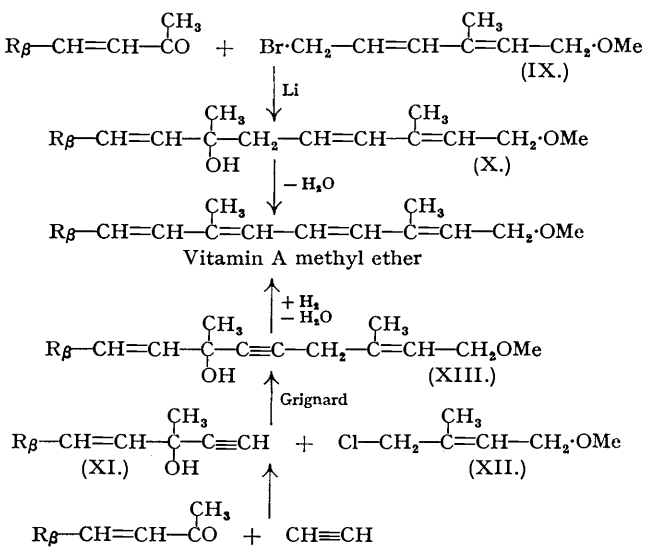
Reformatsky reaction, this ketone behaves anomalously in many ways, notably in condensations involving Grignard reagents (cf. Jones, *Ann. Reports*, 1941, **38**, 180), and this immediately imposes a severe restriction on its potential usefulness.

The first reported synthesis of material showing growth-promoting activity is that of Kuhn and Morris (*Ber.*, 1937, **70**, 853). These workers subjected β-ionone to the usual Reformatsky reaction using ethyl bromoacetate, and obtained ethyl β-ionylideneacetate (III). This was



converted into its *o*-toluidide (IV) and thence into the chloro-imide (V) by phosphorus pentachloride in ether. Reduction of (V) with chromous chloride yielded the *o*-tolil of β-ionylideneacetaldehyde (VI) from which the free aldehyde (VII) was obtained by hydrolysis with oxalic acid. β-Ionylideneacetaldehyde was found to condense with β-methylcrotonaldehyde in the presence of piperidine acetate to yield a product (VIII) which on reduction with aluminium *iso*propoxide furnished a mixture of alcohols which, after partial purification by adsorption on alumina, showed on bioassay a vitamin A content of approximately 5%. Unfortunately, this synthesis has not been found to be reproducible by other workers (Karrer and Ruegger, *Helv. Chim. Acta*, 1940, **23**, 284; Krauze and Slobodin, *J. Gen. Chem. Russia*, 1940, **10**, 907).

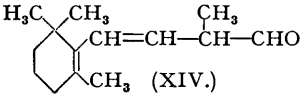
In 1939 Kipping and Wild claimed to have synthesised vitamin A methyl ether by condensing β-ionone with the bromo-ether (IX), using lithium instead of magnesium (*Chem. and Ind.*, 1939,

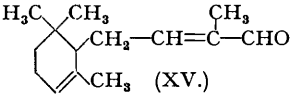


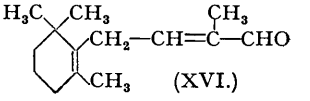
58, 802), but this claim has not been substantiated. Still more recently Oroshnik (*J. Amer. Chem. Soc.*, 1945, **67**, 1627) has published a preliminary note describing the synthesis of vitamin

A methyl ether by the condensation of the ethynylcarbinol (XI) derived from β-ionone with 1-chloro-4-methoxy-2-methylbut-2-ene (XII) followed by semihydrogenation and dehydration. The position of the absorption maximum of the final product, however, is at 3150 A. instead of at 3250 A. as would be expected. This would appear to be due, at least in part, to the presence of a compound containing only four conjugated double bonds. In a private communication, however, Dr. Oroshnik informs me that his product possesses biological activity.

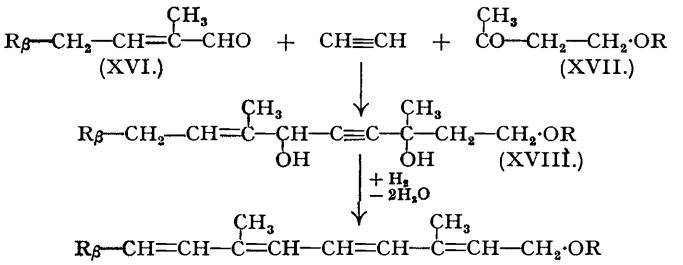
The unsatisfactory and restricted nature of the direct route from β-ionone led us in 1939 to search for more fruitful starting materials. My attention was drawn by my former research assistant, W. E. Jones, to a paper by Ishikawa and Matsuura (*Chem. Zentr.*, 1937, II, 3452) in which what was said to be β-ionone was subjected to a Darzens reaction with ethyl chloroacetate, and the glycide ester so obtained saponified and decarboxylated to give the aldehyde (XIV). We have shown that in fact the aldehyde obtained by these authors must have been the α-ionone derivative (XV) which, as such, is obviously useless for further relevant synthetic work. A complete reinvestigation of the reaction was then carried out using carefully purified β-ionone regenerated from its crystalline semicarbazone, and we finally succeeded in obtaining the αβ-unsaturated C_{14}-aldehyde (XVI), which in contrast to β-ionone reacts normally with Grignard


(XIV.)


(XV.)

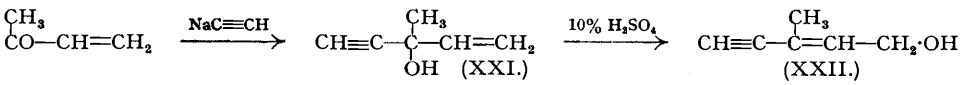

(XVI.)

type reagents. In particular we showed that it condensed readily with sodium acetylide in liquid ammonia and with other acetylenes by the Grignard method to give the normal carbinols (*e.g.*, XXVI) (Heilbron, Johnson, Jones, and Spinks, *J.*, 1942, 727; Cymerman, Heilbron, Jones, and Lacey, *J.*, 1946, 500). The success of this reaction led us to adumbrate the following basic scheme for the synthesis of vitamin A :



The three components—the C_{14}-aldehyde (XVI), acetylene, and a ketobutanol derivative (XVII)—should be capable of being condensed together to yield a product (XVIII), containing the full carbon skeleton of vitamin A, which on semihydrogenation and dehydration would be expected to furnish vitamin A or a simple derivative. There are obviously many permutations and combinations of the methods by which the condensations might be achieved, and it was our intention to make a systematic study of all the likely possibilities. The impact of war, however, severely curtailed our normal research activities after 1940, and we were further hampered by a difficulty in obtaining pure β-ionone which continued until 1946. Accordingly, we decided to embark on a detailed investigation of the main reaction involved, namely, the study of the condensation of αβ-unsaturated carbonyl compounds both with acetylene and with substituted ethynyl derivatives of all types, a subject that had hitherto received scant attention in the literature. This work has led us into many rich and hitherto unexplored pastures of organic chemistry, and a number of novel and useful reactions has been brought to light. One of the most striking of these is the rearrangement which may be formulated in the following manner :

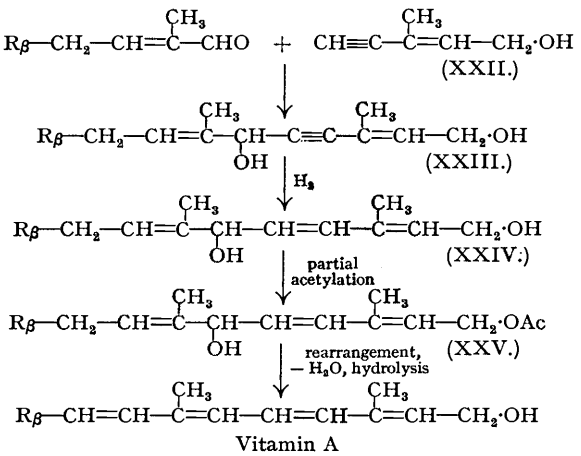

(where R_1, R_2, R_3, R_4 = H or alkyl.)



Structure (XIX) represents the general formulation for an acetylenic carbinol derived from a conjugated polyene carbonyl compound. We have demonstrated that when these compounds are treated with acids a smooth anionotropic rearrangement takes place and the polyene system moves into conjugation with the triple bond to yield fully conjugated compounds of the type (XX) in almost quantitative yields (Jones and McCombie, *J.*, 1943, 261; Heilbron, Jones, *et al.*, *J.*, 1943, 264, 265, 268; 1944, 134, 136, 140, 141, 144; 1945, 27, 77, 90; 1946, 500, 937). We also successfully accomplished the semihydrogenation of the unrearranged acetylenic carbinols of type (XIX) and found that the corresponding ethylenic carbinols rearrange even more easily to yield the fully conjugated polyene carbinols (Heilbron, Jones, McCombie, and Weedon, *J.*, 1945, 84). It was also found that, if the rearrangement be conducted in a solution of an alcohol, the corresponding rearranged ether is obtained (Heilbron, Jones, and Weedon, *J.*, 1945, 81; Heilbron, Jones, McCombie, and Weedon, *J.*, 1945, 88).

Among the compounds we subjected to this reaction was methyl vinyl ketone. This was condensed in normal fashion with sodium acetylide to yield the ethynyl carbinol (XXI) which smoothly isomerised with dilute sulphuric acid to the primary alcohol, 3-methylpent-2-en-4-yn-1-ol (XXII) (Cymerman, Heilbron, and Jones, *J.*, 1945, 90). This compound is obviously a key intermediate in the synthesis of vitamin A as condensation with the C_{14}-aldehyde (XVI) should yield directly a compound containing the full carbon skeleton of vitamin A (Cymerman, Heilbron, Johnson, and Jones, *J.*, 1944, 141). As, however, no C_{14}-aldehyde was then available, we regretfully had to relegate the project to some post-war date and continue with our fundamental, though less spectacular, pioneer work.

Meantime, Isler and his collaborators in Switzerland working in the Hoffman–La Roche laboratories have recently announced the successful synthesis of vitamin A on the above lines, thus confirming the validity of our proposals (*Helv. Chim. Acta*, 1947, **30**, 1911).



The method employed by these authors was to condense the C_{14}-aldehyde with methylpentenynol (XXII) in the normal Grignard manner to obtain the acetylenic carbinol (XXIII), semihydrogenation of which employing a partly poisoned palladium catalyst yielded the polyene glycol (XXIV). The primary hydroxyl group was protected by selective acetylation, and the resulting ester (XXV) treated with a solution of iodine in light petroleum, whereby rearrangement accompanied by simultaneous dehydration occurred giving crude vitamin A acetate. Hydrolysis and purification of the product *via* the anthraquinonecarboxylate yielded crystalline vitamin A identical in every respect with the natural product.

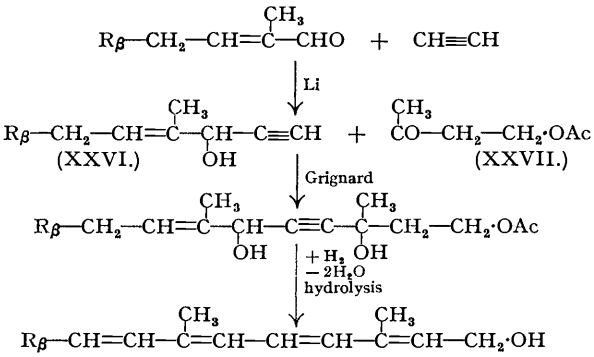
In the same way, using the methyl ester of (XXII), vitamin A methyl ether was obtained; this was found to be nearly as biologically active as the alcohol itself (Isler, Huber, Ronco, and Kofler, *Experientia*, 1946, **2**, 31; Emil Barell Jubilee Volume, Hoffmann-La Roche and Co., Basle, 1946, p. 31).

It should be pointed out that the large-scale production of the synthetic vitamin by this process has only been made possible by the discovery by Isler and his colleagues that the intermediate glycide ester is extremely sensitive to heat and that, therefore, its saponification to the acid must be carried out at about 5°. It was further observed by these workers that under

these precise conditions the acid immediately loses carbon dioxide giving the aldehyde, which, after distillation, is obtained pure in 80% yield, as against the 10—20% previously recorded.

As so often happens, Milas in America seems to have hit on the idea of using the C_{14}-aldehyde for the synthesis of vitamin A about the same time as we did, and in a number of patents has recorded the preparation of materials containing some vitamin A both by a method similar to that adopted by the Swiss workers, and alternatively by condensation of the ethynyl carbinol (XXVI) with ketobutanol acetate (XXVII) followed by the obvious procedures of semihydrogenation, dehydration, and saponification. As this author's work has, apart from a short note, so far only been recorded in a series of patents (U.S.PP. 3,369,156—157; 3,369,166—168; 2,382,085—086; *Science*, 1946, **103**, 581) there is a lack of precise information which is unfortunate, as it renders the work difficult to assess, especially as the C_{14}-aldehyde is regarded as having the $\beta\gamma$-structure (XIV).

I wish now to devote a few minutes to the consideration of still another novel approach to synthetic vitamin A. By the use of *N*-bromosuccinimide (Ziegler *et al.*, *Annalen*, 1942, **551**, 80), methyl γ-bromocrotonate has become readily available, and its use in the Reformatsky reaction when applied to β-ionone has had fruitful results. This reaction has been studied



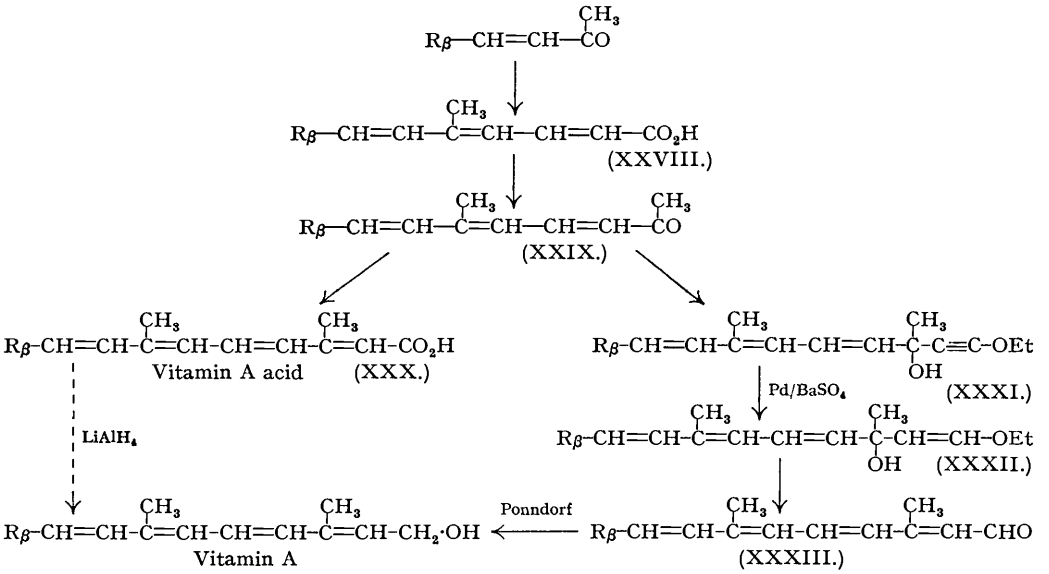
Vitimin A.

contemporaneously by Arens and Van Dorp in Holland (*Rec. Trav. chim.*, 1946, **65**, 338), by Karrer in Switzerland (Karrer, Jucker, and Schick, *Helv. Chim. Acta*, 1946, **29**, 704), and in my own laboratory (Heilbron, Jones, and O'Sullivan, *J.*, 1946, 866), and leads to the crystalline C_{17}-acid (XXVIII). Attempts both on our part and by Karrer and Schwyzer (*Helv. Chim. Acta*, 1946, **29**, 1191) to prepare the corresponding polyene C_{19}-acid with five conjugated ethenoid linkages which we anticipated might have biological activity, by condensing methyl ω-bromosorbate with β-ionone, have been entirely abortive. The next stage, the conversion of the C_{17}-acid to the C_{18}-ketone (XXIX) was handled somewhat differently by each group of workers. In my laboratory the C_{17}-acid chloride was prepared and brought into reaction with dimethylcadmium; Karrer also proceeded *via* the acid chloride, employing zincmethyl iodide as the reactant. The Dutch workers, however, using methyl-lithium (cf. Gilman and van Ess, *J. Amer. Chem. Soc.*, 1933, **55**, 1258), have proceeded directly from the C_{17}-acid to the C_{18}-ketone which they then condensed with ethyl bromoacetate. Hydrolysis of the resultant C_{20}-ester gave " vitamin A acid " in the form of yellow crystals exhibiting maximal absorption at 3470 A. The importance of this synthesis springs from the observation that the sodium salt of the C_{20}-acid, buffered to pH 10, is as biologically active as vitamin A itself. It is also obvious that the application to it of the elegant, recently discovered reagent, lithium aluminium hydride (Nystrom and Brown, *J. Amer. Chem. Soc.*, 1947, **69**, 1197), should yield vitamin A directly (cf. Milas and Harrington, *ibid.*, p. 2247).

Arens and van Dorp have further utilised the C_{18} ketone (XXIX) in an elegant synthesis of vitamin A itself (*Nature*, 1947, **160**, 189). They condensed it with ethoxyacetylene (Jacobs, Cramer, and Hanson, *J. Amer. Chem. Soc.*, 1942, **64**, 223) to obtain the acetylenic carbinol (XXXI) which on semihydrogenation yielded the enol ether (XXXII). Anionotropic rearrangement of this compound under the influence of dilute hydrochloric acid furnished vitamin A aldehyde (XXXIII) which, on reduction by the Ponndorf method, furnished an oil containing about 35% of vitamin A.

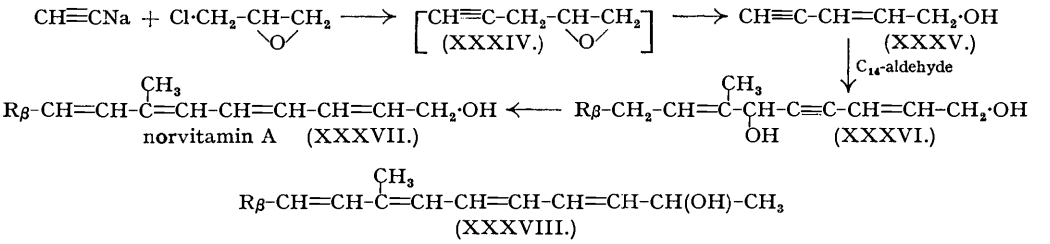
At Imperial College we have now embarked on a comprehensive research programme aimed

at producing modified vitamin A structures. Our general method consists in introducing methyl groups one by one into a basic vitamin A structure completely devoid of all such groups. The



study of the biological activity of these homologous compounds should give a final picture of the mode of dependency of physiological activity on the detailed structure, while at the same time the light absorption exhibited by these compounds should be of considerable interest. I will now give a brief account of some of the reactions we are employing to attain this objective.

The very interesting and useful acetylenic carbinol, pent-2-en-4-yn-1-ol (XXXV), first prepared in my laboratory by a rather lengthy process (Heilbron, Jones, Lacey, McCombie, and Raphael, *J.*, 1945, 77), is now readily available as a result of our observation that it is, rather unexpectedly, the product of the interaction of sodium acetylide and epichlorohydrin in liquid ammonia (Haynes, Heilbron, Jones, and Sondheimer, *J.*, 1947, 1583). This carbinol has now been condensed with the C_{14}-aldehyde (XVI) to give the glycol (XXXVI) from which norvitamin A (XXXVII) has been prepared as a 25% concentrate which exhibits biological activity. A similar condensation has been carried out with the homologous compound hex-3-en-5-yn-2-ol (Jones and McCombie, *J.*, 1943, 261) with the purpose of obtaining (XXXVIII), a modified vitamin A containing a secondary hydroxyl group.

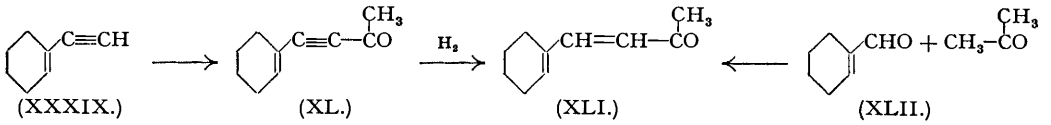


Rβ-CH=CH-C=CH-CH=CH-CH=CH-CH(OH)-CH3
(XXXVIII.)

Obvious key intermediates for our project are the various demethyl-β-ionones, and various methods have been worked out for their preparation. I will illustrate some of these routes using as the example the completely demethylated β-ionone (XLI.).

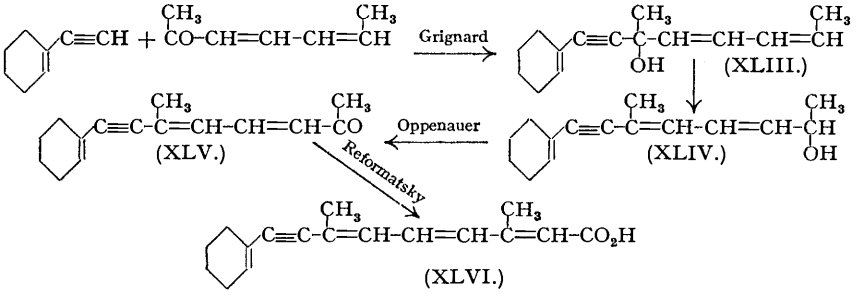
The hitherto ill-defined compound, ethynyl*cyclo*hexene (XXXIX), is now available in quantity as a result of our observation that the acetylene carbinol of *cyclo*hexanone can be dehydrated smoothly over aluminium phosphate. The Grignard complex of this hydrocarbon has been treated with acetic anhydride to furnish the ketone (XL), semihydrogenation of which yielded (XLI). An alternative direct synthesis of (XLI) based on the condensation of *cyclo*-hexenealdehyde (XLII), which has also been made readily available, with acetone has also been

achieved. The resulting demethyl-β-ionone and its homologues are being subjected to reactions similar to those already discussed to furnish the corresponding demethylvitamins.



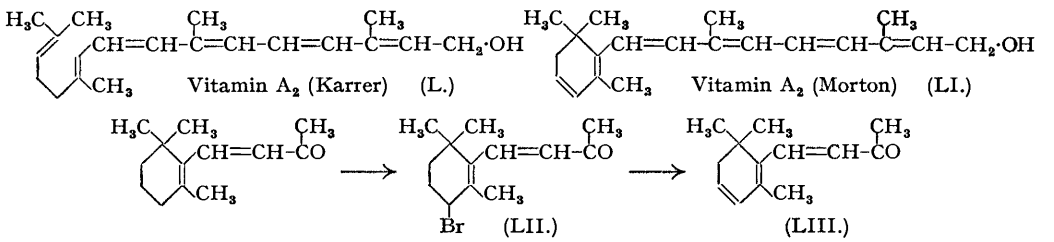
(XXXIX.) (XL.) (XLI.) (XLII.)

Ethynyl*cyclo*hexene is also the starting material for a synthesis providing a new general method of obtaining lower homologues of vitamin A acid and hence of vitamin A itself. We have condensed the hydrocarbon with the easily obtainable crotonylideneacetone to furnish the carbinol (XLIII) which smoothly undergoes anionotropic rearrangement to the isomeric secondary carbinol (XLIV), which, when oxidised by the Oppenauer method, yields the ketone (XLV). Treatment of this ketone with methyl bromoacetate under Reformatsky conditions readily leads to the crystalline C_{17}-acid (XLVI), analogous to vitamin A acid. This is of considerable interest as it exhibits definite although weak physiological activity. The partial hydrogenation of this acid to produce the modified vitamin A acid itself is now being studied.



The accomplishment of the synthesis of vitamin A, or A_1 as it should strictly be designated, has brought into sharp focus the question of the constitution of vitamin A_2, the vitamin associated with fresh-water fish (Gillam, Heilbron, Jones, and Lederer, *Biochem. J.*, 1938, **32**, 405). The structure of this compound has not so far been definitely settled. Karrer and his co-workers, mainly on the basis of the production of acetone on ozonolysis and the absorption maximum at 3450 A., have put forward the open chain formula (L) (*Helv. Chim. Acta*, 1941, **24**, 161E; 1942, **25**, 1650; 1943, **26**, 1758). This formulation, however, fails to explain a large number of facts, among which may be mentioned the circumstance that lycopene, unlike β-carotene, fails to function as a provitamin A_2 (Morton and Creed, *Biochem. J.*, 1939, **33**, 318). Morton, Salah, and Stubbs, on the other hand, consider that vitamin A_2 possesses the cyclic structure (LI), being thus a dehydrovitamin A (*Nature*, 1947, **159**, 744).

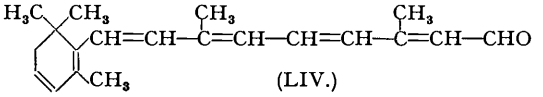
In my laboratory we are now engaged in the attempted synthesis of (LI) and the obvious starting material, dehydro-β-ionone (LIII) has been prepared by Dr. Henbest as follows. β-Ionone was treated with *N*-bromosuccinimide to give the bromo-compound (LII) which, on dehydrobromination with diethylaniline yielded dehydro-β-ionone (LIII) ($\lambda_{max.}$ 3370 A.) directly. This is now being subjected to the procedures already described in the synthesis of vitamin A_1.



A relatively minor symptom of vitamin A deficiency is night blindness, and the fundamental relationship connecting the metabolism of vitamin A and related carotenoids with the biochemical processes in the retinal rods and cones has been the subject of intensive research. One essential stage in the processes of dark-adaptation in the eyes of vertebrates is the

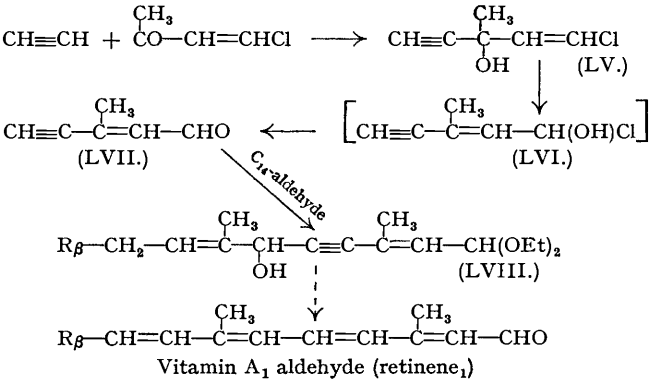
breakdown and regeneration of visual purple in the retinal rods. The visual purple complex obtained from these vertebrates utilising vitamin A_1 in the metabolic processes is a protein termed rhodopsin, the prosthetic group of which, originally believed to be a carotenoid, retinene, has recently been shown to be identical with vitamin A_1 aldehyde (Morton *et al.*, *Nature*, 1944, **153**, 69, 405).

Similarly, the visual purple from vertebrates utilising vitamin A_2 is a closely related protein, porphyropsin, the prosthetic group of which, retinene$_2$, has been shown to be identical with vitamin A_2 aldehyde, which is believed to be (LIV) (Morton, Salah, and Stubbs, *Nature*, 1947, **159**, 744) previously obtained in my laboratory by oxidation of vitamin A_1 with aluminium *tert.*-butoxide in the presence of diethyl ketone (Haworth, Heilbron, Jones, Morrison, and Polya, *J.*, 1939, 128).



(LIV.)

We are also working on the synthesis of vitamin A_1 aldehyde by a new and interesting route. The vital intermediate is the highly reactive aldehyde (LVII), recently prepared by the following method (Jones and Weedon, *J.*, 1946, 938). Methyl chlorovinyl ketone was condensed with sodium acetylide to give the chlorocarbinol (LV), which in the presence of dilute acids rearranged to give (LVII) directly, *via* the hypothetical intermediate (LVI). The diethylacetal of this has been prepared, and by condensation with C_{14}-aldehyde followed by the procedure already described it should yield vitamin A_1 aldehyde.



Vitamin A_1 aldehyde (retinene$_1$)

And so, after many years, victory has come, and the romance of exploration, of high hopes and bitter disappointment, will in a few years simply be recorded in the text-books of organic chemistry in a few terse sentences. Looking back, I still can recapture the excitement of the early days of investigation with Morton and W. E. Jones in Liverpool, with Gillam in Manchester, and with E. R. H. Jones in London. To each of these and to a host of loyal and enthusiastic research students, especially Dr. Raphael and Mr. Sondheimer, I owe a debt of gratitude for their generous help and co-operation.