Carotenoids and Related Compounds. Part XV.[1] The Structure and Synthesis of Phytoene, Phytofluene, ζ-Carotene, and Neurosporene

By J. B. Davis, L. M. Jackman, P. T. Siddons, and B. C. L. Weedon

Structures have been established for the polyenes, phytoene, phytofluene, ζ-carotene and neurosporene by spectral studies and synthesis. The stereochemistry of these polyenes is discussed.

It is now generally accepted [2,3] that phytoene, phytofluene, ζ-carotene, and neurosporene are intermediates in the biosynthesis of carotenoids, rather than artefacts or by-products of this process.[4,5] Each is believed to be converted into the next member of the series by a dehydrogenation which introduces an extra double bond and extends the conjugated system by two double bonds.[2,3] As a contribution to the solution of

[1] Part XIV, J. W. K. Burrell, R. F. Garwood, L. M. Jackman, F. Oskay, and B. C. L. Weedon, preceding Paper.

[2] S. L. Jensen, G. Cohen-Bazire, and R. Y. Stanier, *Nature*, 1961, **192**, 1168.

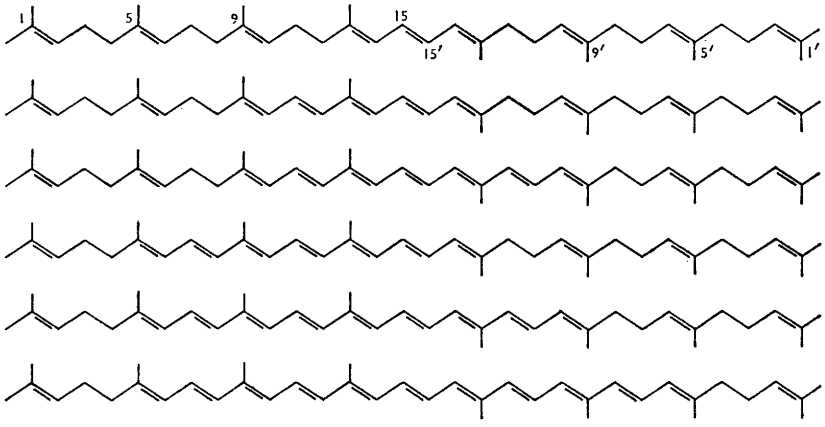
[3] For some recent reviews see: J. W. Porter and D. G. Anderson, *Arch. Biochem. Biophys.*, 1962, **97**, 520; T. W. Goodwin, "The Biosynthesis of Vitamins and Related Compounds," Academic Press, London, 1963; C. O. Chichester and T. O. M. Nakayama "Biogenesis of Natural Compounds," ed. P. Bernfeld, Pergamon Press, Oxford, 1963; T. W. Goodwin "Chemistry and Biochemistry of Plant Pigments," ed. T. W. Goodwin, Academic Press, London, 1965; J. A. Olsen, *J. Lipid Research*, 1964, **5**, 281.

[4] T. W. Goodwin, "The Comparative Biochemistry of the Carotenoids," Chapman and Hall, London, 1952.

[5] G. Mackinney, *Ann. Rev. Biochem.*, 1952, **21**, 473; A. E. Purcell, *Arch. Biochem. Biophys.*, 1964, **105**, 606.

this problem we undertook to resolve the doubts which existed concerning the structure of the polyenes, since a number of the formulæ that have been entertained were difficult to reconcile with any rational biosynthetic scheme, or suggested a much more complex situation than that which has now emerged. An outline of our main results was published in 1961.[6]

Of the four polyenes, only neurosporene has been crystallised,[7] the first two members of the series being viscous oils [8,9] and (all-*trans*-) ζ-carotene a low melting solid (see Experimental section). The instability, particularly in air, and inaccessibility of these compounds have greatly handicapped previous structural studies. However, the elegant work of Rabourn and Quackenbush [10] provided strong grounds for formulating phytoene as (I) even though the evidence for some features, notably the central location of the conjugated triene unit, was not conclusive.



(I)

(II)

(III*a*)

(III*b*)

(IV)

(V)

Structure (II) for phytofluene was proposed by Zechmeister [11] because treatment of phytoene with *N*-bromosuccinimide had been shown [12] to give a product which was spectrally and chromatographically indistinguishable from natural phytofluene. Similar dehydrogenations of other members of the series, leading ultimately to lycopene (V), have been reported.[12] However, some of the products may conceivably have been mixtures of positional isomers due to dehydrogenation at alternative sites, or to rearrangement of the polyene systems in the allylic intermediates. Degradative studies by Rabourn and Quackenbush [13] are consistent with structure (II) for phytofluene.

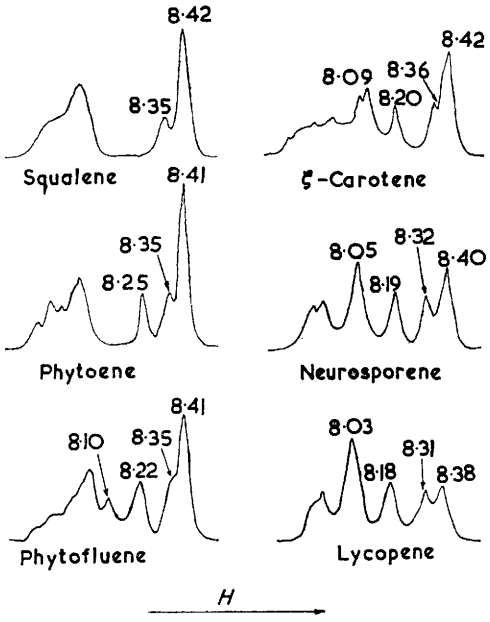
Nash, Quackenbush, and Porter [14] regarded ζ-carotene as an octahydrolycopene, but in a report which is not generally available Rabourn and Quackenbush [15] favoured the tetrahydro-lycopene structure (III*a*); the evidence cited was, however, equally compatible with the unsymmetrical isomer (III*b*).

The tetrahydro-lycopene (VII*a*) was synthesised by Karrer *et al.*[16] and reported to have spectral and chromatographic properties indistinguishable from those of natural neurosporene; it was suggested that the observed difference in melting point may have been due to stereoisomerism involving asymmetric centres at C-5 and C-5′. Structure (VII*a*) has been accepted by some workers for neurosporene, and its apparent confirmation by synthesis has lent support to analogous structures, also embodying methyl groups attached to tetrahedral (sp^3) carbon atoms, for the other polyenes.[16,17] Rabourn [18] has pointed out that the dihydro-lycopene structure (IV) would be more in accord with the biosynthetic role envisaged for neurosporene as an intermediate between ζ-carotene and lycopene, but no direct support for this suggestion was available.

Examination of the nuclear magnetic resonance (n.m.r.) spectra of the four polyenes immediately disposes of all structures such as (VII*a*) with methyl groups on sp^3 carbon atoms. Unlike (VII*b*), which was synthesised by reaction of crocetindial [19] (VI) with the appropriate Wittig reagent, they do not absorb in the region τ 9·0—9·15 where bands due to "paraffinic" methyls are

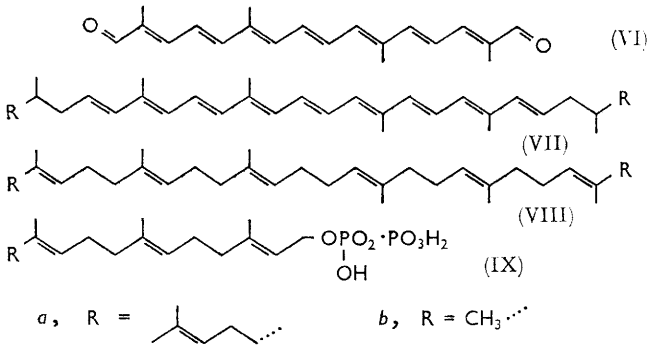
[6] J. B. Davis, L. M. Jackman, P. T. Siddons, and B. C. L. Weedon, *Proc. Chem. Soc.*, 1961, 261.
[7] F. Haxo, *Arch. Biochem.*, 1949, **20**, 400.
[8] J. W. Porter and F. P. Zscheile, *Arch. Biochem.*, 1946, **10**, 547; J. W. Porter and R. W. Lincoln, *ibid.*, 1950, **27**, 390.
[9] L. Zechmeister and A. Polgár, *Science*, 1944, **100**, 317; L. Zechmeister and A. Sandoval, *Arch. Biochem.*, 1945, **8**, 425.
[10] W. J. Rabourn and F. W. Quackenbush, *Arch. Biochem. Biophys.*, 1956, **61**, 111; W. J. Rabourn, F. W. Quackenbush, and J. W. Porter, *ibid.*, 1954, **48**, 267.
[11] L. Zechmeister, *Progr. Chem. Org. Nat. Prod.*, 1958, **15**, 31.
[12] B. K. Koe and L. Zechmeister, *Arch. Biochem. Biophys.*, 1952, **41**, 236; L. Zechmeister and B. K. Koe, *J. Amer. Chem. Soc.*, 1954, **76**, 2923.
[13] W. J. Rabourn and F. W. Quackenbush, Journal Paper No. 1111 of the Purdue University Agricultural Experiment Station.
[14] H. A. Nash, F. W. Quackenbush, and J. W. Porter, *J. Amer. Chem. Soc.*, 1948, **70**, 3613.
[15] W. J. Rabourn and F. W. Quackenbush, Journal Paper No. 1116 of the Purdue University Agricultural Experiment Station.
[16] C. H. Eugster, E. Linner, A. H. Trivedi, and P. Karrer, *Helv. Chim. Acta*, 1956, **39**, 690.
[17] T. W. Goodwin, *J. Sci. Food Agric.*, 1953, **5**, 209; *Adv. Enzymol.*, 1959, **21**, 295; E. C. Grob "Biosynthesis of Terpenes and Sterols," Churchill, London, 1959; G. Mackinney "Metabolic Pathways," ed. D. M. Greenberg, Academic Press, New York, 1960.
[18] W. J. Rabourn, Paper presented before the Division of Biological Chemistry, 132nd Meeting Amer. Chem. Soc., 1957; cf. Y. Mase, *J. Vitaminol.*, 1959, **5**, 161; A. Winterstein, *Angew. Chem.*, 1960, **72**, 902.
[19] O. Isler, H. Gutmann, H. Lindlar, M. Montavon, R. Rüegg, G. Ryser, and P. Zeller, *Helv. Chim. Acta*, 1956, **39**, 463.

found.[20] However, all four compounds exhibited strong bands that can be ascribed to methyls of the "in-chain," "end-of-chain," and "olefinic" types.* Although the various bands were not fully resolved



N.m.r. spectra of the natural hydro-lycopenes and related compounds (in CCl_4 or $CDCl_3$, 56·4 Mc./sec.)

(at 56·4 Mc./sec.), comparison of the spectra indicates that each polyene has one less "olefinic" methyl, and one more "in-chain" methyl than its more saturated bands at τ 8·42 and 8·35 in the proportions of 3 : 1. Since squalene is known[22] to possess the all-*trans*-configuration, these bands may be assigned to the olefinic methyl groups which are, respectively, *trans* and *cis* to the hydrogen atom at $C_{(\beta)}$ on the (unconjugated)trisubstituted double bonds ($-CH_2\cdot C_{(\alpha)}Me{:}C_{(\beta)}H\cdot CH_2-$). Further support for this interpretation comes from a consideration of the spectra



of the farnesols and related compounds.[1,21] It can therefore be concluded from the relative intensities of the olefinic methyl bands in phytoene, phytofluene, ζ-carotene, and neurosporene, that all non-terminal, unconjugated double bonds in these polyenes also have the *trans*-configuration.[6] (The stereochemistry of the conjugated systems is discussed later.) This conclusion is in keeping with the view[3,17] that the biosynthesis of the (C_{40}-) polyenes involves the initial formation of a (C_{20}-) geranyl-geranyl derivative (*e.g.* IX*a*) by a route

Nuclear magnetic resonance properties *

	Methyls on polyene chain		Methyls on isolated double bonds		Methyls on sp^3 carbon atoms
	In-chain	End-of-chain	cis †	*trans* †	
Squalene (VIIIb) ‡	—	—	8·35	8·42	—
Phytoene (I), natural	—	8·25	8·35	8·41	—
,, synthetic	—	8·25	8·34	8·43	—
C_{30}-model (XII)	—	8·23	8·31	8·39	—
Phytofluene (II), natural	8·10	8·22	8·35	8·41	—
,, synthetic	8·12	8·23	8·36	8·42	—
ζ-Carotene (III*a*), natural	8·09	8·20	8·36	8·42	—
,, synthetic	8·09	8·22	8·36	8·43	—
iso-ζ-Carotene (IIIb), synthetic	8·03	8·19	8·32	8·39	—
C_{30}-model (XIII)	8·01	8·19	8·33	8·39	—
Neurosporene (IV), natural	8·05	8·19	8·32	8·40	—
,, synthetic	8·06	8·19	8·35	8·40	—
Hydrocarbon (VII*b*)	8·08	—	—	—	9·10 ¶
Lycopene (V) §	8·03	8·18	8·31	8·38	—

* The spectra were determined for deuterochloroform or carbon tetrachloride solutions at 56·4 Mc./sec., and calibrated against tetramethylsilane as an internal standard. Methyl bands only are given; the relative intensities of the bands were consistent with the proposed structures. † With respect to the hydrogen atom at $C_{(\beta)}$ in tri-substituted double bonds, $-CH_2\cdot C_{(\alpha)}Me{:}C_{(\beta)}H\cdot CH_2-$. ‡ Cf. Bates and Gale.[21] ¶ Doublet (J = 7 c./sec.). § Barber *et al.*[20]

precursor (Figure and Table). The slight change in the position of the "end-of-chain" methyl band from τ 8·25 to 8·18 as the conjugated chain is lengthened is not unexpected.

As has also been noted by Bates and Gale,[21] the n.m.r. spectrum of squalene † (VIII*b*) exhibits methyl analogous to that leading to (C_{15}-) farnesyl pyrophosphate (IX*b*) in the biosynthesis of (C_{30}-) squalene (VIII*b*). There is still lack of agreement as to whether the initial C_{40}-compound in the biosynthesis of carotenoids

* The classification of methyl groups, and band assignments, have been discussed previously.[6, 20]

† The authors thank Dr. D. Arigoni (Zürich) for a sample of all-*trans*-squalene.

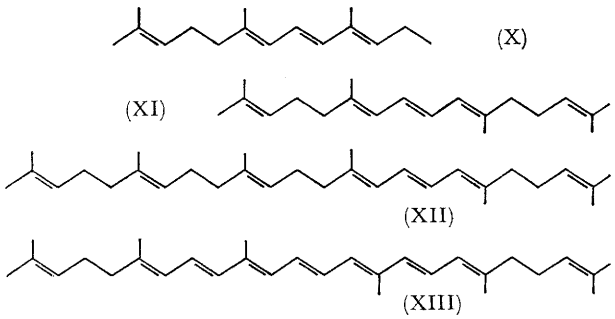
[20] M. S. Barber, J. B. Davis, L. M. Jackman, and B. C. L. Weedon, *J. Chem. Soc.*, 1960, 2870.

[21] R. B. Bates and D. M. Gale, *J. Amer. Chem. Soc.*, 1960, **82**, 5749; R. B. Bates, D. M. Gale, and B. J. Gruner, *J. Org. Chem.*, 1963, **28**, 1086.

[22] N. Nicholaides and F. Laves, *J. Amer. Chem. Soc.*, 1954, **76**, 2596.

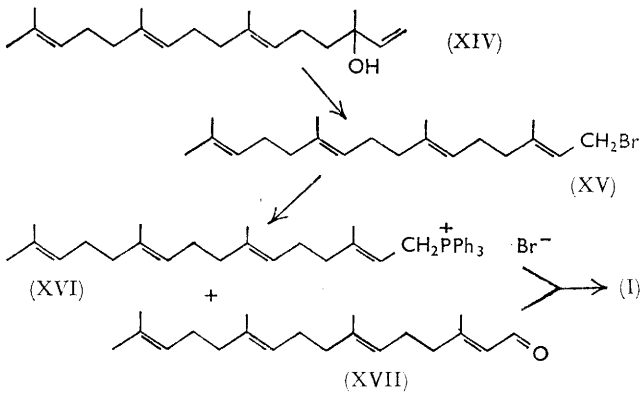
is a C_{40}-analogue of squalene, [" lycopersene," (VIII*a*)] or a conjugated polyene such as phytoene,[23] but the enzymatic transformation of farnesyl pyrophosphate into phytoene has been demonstrated.[23a]

Although the different methyl bands observed, and their relative intensities, provide strong support for formulating phytoene, phytofluene, and neurosporene as (I), (II), and, (IV), respectively, it is not possible to distinguish (on the basis of the n.m.r. results) between (III*a*) and (III*b*) for ζ-carotene; since both alternatives have the same types and relative numbers of methyls, they would be expected to exhibit very similar spectra. Moreover, the conclusions drawn regarding the location of the conjugated systems in phytoene and phytofluene are based on the assumption that bands due to " end-of-chain " methyls ($-CH_2\cdot CMe{:}CH\cdot CH{:}CH-$) can be distinguished from those due to " isoprenoid " methyls ($-CH{:}CH\cdot CMe{:}CH\cdot CH_2-$). It was therefore disquieting to find that the bands due to these two types of methyl cannot be resolved (at 56·4 Mc./sec.) in the spectrum of (X). Final proof of the structures of the four polyenes was therefore sought by synthesis. In developing



suitable routes, the C_{20}- and C_{30}-models (XI) and (XII) of phytoene, and the C_{30}-model (XIII) of ζ-carotene were also prepared (see Experimental section).

Phytoene (I).—Treatment of *trans*-geranyl-linalool [24] (XIV) with phosphorus tribromide gave geranyl-geranyl bromide (XV), which was converted into the corresponding triphenylphosphonium bromide (XVI). The product isolated by repeated crystallisation of the crude salt was assumed to have the all-*trans*-configuration. Geranyl-geranyl bromide (XV) was also converted by the nitropropane route [25] into geranyl-citral (XVII); its n.m.r. spectrum indicated an all-*trans*-structure, but the presence of about 10% of a *cis*-isomer was revealed by gas–liquid chromatography (g.l.c.). A Wittig reaction between the two C_{20}-components (XVI) and (XVII) furnished the required hydrocarbon (I). Its infrared (i.r.) absorption properties showed the presence of both *cis*-15- and *trans*-15-isomers, and it is possible from the mode of synthesis that *cis*-13-isomers were also present. A partial concentration of the all-*trans* form was achieved by means of the thiourea inclusion compound.



The synthetic mixture exhibited ultraviolet (u.v.) absorption maxima at the same wavelengths as natural phytoene (though of slightly greater intensity). A non-central location of the conjugated triene unit in phytoene can now be excluded as this would result in the same chromophore as is present in (X), which absorbs at *ca.* 7 mμ shorter wavelengths. This argument is not vitiated by any differences in geometrical configuration since these influence mainly the intensity, rather than the positions, of absorption in the conjugated trienes. As expected, the methyl bands in the n.m.r. spectra of natural phytoene and the synthetic mixture were identical.

Natural phytoene was tentatively assigned [10] a *cis*-15-structure on the basis of its i.r. absorption maximum at 758 cm.$^{-1}$. Its inability to form a thiourea complex is consistent with this formulation.[10] The properties of the synthetic mixture substantiate this assignment. The only structural feature still to be elucidated is the configuration of the double bonds at C-13 and C-13′. On biogenetic grounds these are probably *trans*. The same conclusion was recently drawn by Jungalwala and Porter.[25a] These authors reported that stereomutation of natural phytoene gives a product similar to one synthesised by our route.

Phytofluene (II).—Reaction of *trans*-nerolidol [24] (XVIII) with phosphorus tribromide gave farnesyl bromide (XIX) which was converted by the nitropropane route [25] into farnesal (XX). Condensation of the latter with the phosphonate (XXI), prepared from methyl ω-bromosenecioate, gave the ester (XXII) which was reduced with lithium aluminium hydride to the alcohol (XXIII). Oxidation with manganese dioxide gave the corresponding aldehyde (XXIV) which reacted with the Wittig reagent from (XVI) to give the required hydrocarbon (II). The all-*trans*-isomer was isolated by

[23] E. C. Grob, K. Kirschner, and F. Lynen, *Chimia* (*Switz.*), 1961, **15**, 308; E. C. Grob and A. Boschetti, *ibid.*, 1962, **16**, 15; D. G. Anderson and J. W. Porter, *Arch. Biochem. Biophys.*, 1962, **97**, 509; B. H. Davies, D. Jones, and T. W. Goodwin, *Biochem. J.*, 1963, **87**, 326; E. Nusbaum-Cassuto and J. Villoutreix, *Compt. rend.*, 1965, **260**, 1013.

[23a] F. B. Jungalwala and J. W. Porter, *Plant Physiol.* (Suppl.), 1965, **40**, xviii; cf. D. G. Anderson and J. W. Porter, *Arch. Biochem. Biophys.*, 1962, **97**, 509.

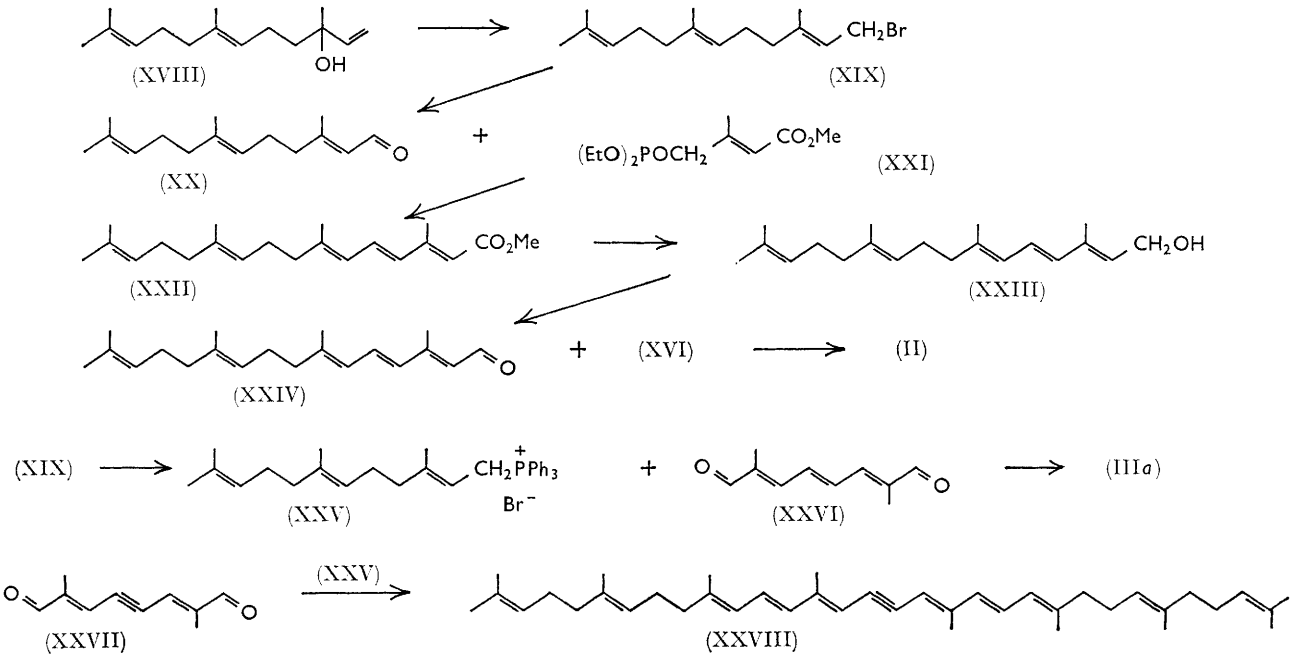
[24] O. Isler, R. Rüegg, L. H. Chopard-dit-Jean, A. Winterstein, and O. Wiss, *Helv. Chim. Acta*, 1958, **41**, 786.

[25] M. Montavon, H. Lindlar, R. Marbet, R. Rüegg, G. Ryser, G. Saucy, P. Zeller, and O. Isler, *Helv. Chim. Acta*, 1957, **40**, 1250.

[25a] F. B. Jungalwala and J. W. Porter, *Arch. Biochem. Biophys.*, 1965, **110**, 291.

chromatography, and was identical in all respects with a sample of all-*trans*-phytofluene prepared from carrot oil. It is of interest to note that "γ-vitamin A" (XXXV), which illustrates the alternative alkylation pattern for conjugated pentaenes in the lycopene series, absorbs at shorter wavelengths than phytofluene.

A number of geometrical isomers of phytofluene have been reported;[13,26] the stereochemistry of the native form is not known (see, however, discussion on ζ-carotene and references 25*a* and 26).



ζ-*Carotene* (IIIa).—Farnesyl bromide (XIX) from *trans*-nerolidol (XVIII) was converted into the triphenylphosphonium bromide (XXV). The product isolated by repeated crystallisation of the crude salt was assumed to have the all-*trans*-configuration. A Wittig reaction with the dimethyloctatrienedial[27] (XXVI) gave the required hydrocarbon (III*a*). The all-*trans* isomer was isolated as a low-melting unstable solid which was identified with ζ-carotene isolated from carrot oil. Both products exhibited the same behavior on iodine-catalysed stereomutation.

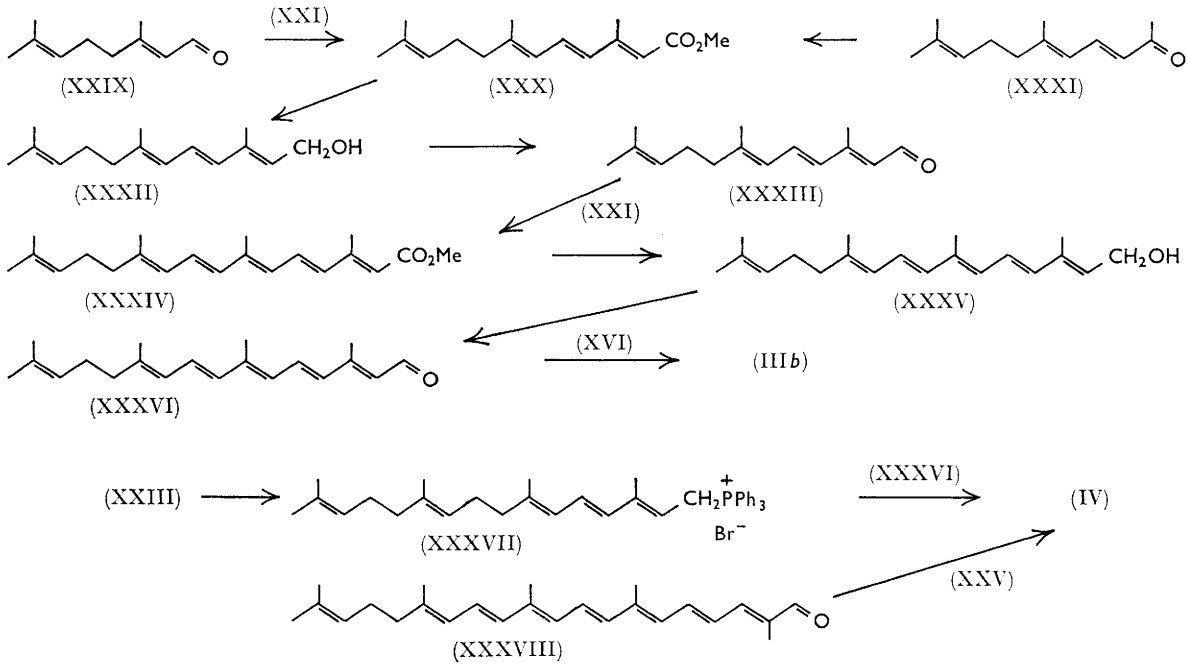
There is some evidence that ζ-carotene occurs in nature as a labile *cis*-form.[15] In view of the identification of phytoene as a *cis*-15-isomer, it seemed desirable to synthesise the *cis*-15-isomer of ζ-carotene. A Wittig reaction of (XXV) with the acetylenic dialdehyde (XXVII)[27] gave crystalline all-*trans*-dehydro-ζ-carotene (XXVIII). Selective hydrogenation over a Lindlar catalyst[28] in the dark gave the required *cis*-15-isomer of ζ-carotene as an unstable oil which exhibited the expected spectral properties. Its light absorption maxima were displaced 3 mμ to shorter wavelengths, compared with the all-*trans*-form, and a strong "*cis*-peak" was apparent in the u.v. region of the spectrum. It had the i.r. absorption band at 777 cm.$^{-1}$, characteristic of *cis*-15-carotenoids.[26] Dr. H. Claes (Tübingen) very kindly compared this central-*cis* product with the *cis*-ζ-carotene which she has observed[29] in chlorella mutants, and which has the same visible and u.v. absorption properties. Although our sample had undergone about 50% stereomutation to the all-*trans*-form in transit, she was able to report that the two isomers in the synthetic material did not separate in mixed chromatograms on alumina from the *cis*- and *trans*-ζ-carotenes, respectively, isolated from chlorella mutants. It is tempting to suggest that the central-*cis* double bond in phytoene is preserved during the dehydrogenation sequence, at least to the ζ-carotene stage, during the biosynthesis of carotenoids in these algæ.

The unsymmetrical structure (III*b*), which was also considered at one stage for ζ-carotene, was synthesised in the following way. Condensation of the phosphonate (XXI) with citral (XXIX) gave the ester (XXX). The same product was also obtained, though in lower yield, by reaction of the corresponding Wittig reagent with citral, and from ψ-ionone (XXXI) by the methoxyacetylene method.[30] Reduction of (XXX) with lithium aluminium hydride gave the alcohol (XXXII) which was oxidised with manganese dioxide to the corresponding aldehyde (XXXIII). Condensation with the phosphonate (XXI) led to the ester (XXXIV) which was

[26] L. Zechmeister, "*cis–trans* Isomeric Carotenoids, Vitamins A, and Aryl Polyenes," Springer-Verlag, Vienna, 1962.
[27] P. Mildner and B. C. L. Weedon, *J. Chem. Soc.*, 1953, 3294; H. H. Inhoffen, O. Isler, G. von der Bey, G. Raspé, P. Zeller, and R. Ahrens, *Annalen*, 1953, **580**, 7.
[28] H. Lindlar, *Helv. Chim. Acta*, 1952, **35**, 446.
[29] H. Claes, *Z. Naturforsch.*, 1961, **16B**, 445.
[30] J. F. Arens and D. A. Van Dorp, *Rec. Trav. chim.*, 1948, **67**, 973.

reduced to "γ-vitamin A" (XXXV).[31] Oxidation with manganese dioxide gave "γ-retinal" (XXXVI)[31] which on treatment with the Wittig reagent from (XVI) furnished the required hydrocarbon (III*b*). Although it did not separate from the symmetrical isomer (III*a*) in mixed chromatograms, it exhibited visible light absorption maxima at wavelengths which were *ca.* 5 mμ shorter, and was therefore clearly different from the solid ζ-carotene which we isolated from carrot oil. Comparable spectral differences have been observed by Dr. B. H. Davies (Aberystwyth) in the "ζ-carotenes" isolated from different natural sources.[32] It is not yet known whether these differences are due to geometrical isomerism, or to positional isomerism of the type now under consideration leading to heptaene systems with different alkylation patterns.

Neurosporene (IV).—Two routes to neurosporene were developed. Reaction of the Wittig reagent (XXXVII) prepared from the C_{20}-alcohol (XXIII) with "γ-retinal" (XXXVI) gave a mixture of geometrical isomers from which the all-*trans*-hydrocarbon (IV) was isolated by chromatography and crystallisation. The same product was obtained more conveniently by reaction of the (C_{25}-) apo-12′-lycopenal[31] (XXXVIII) with the farnesyl Wittig reagent (XXV). The products were identical in all respects with crystalline all-*trans*-neurosporene isolated from *Rhodopseudomonas spheroides*.[33] The only naturally occurring *cis*-isomer of neurosporene so far reported is believed to have a poly-*cis*-configuration.[26]



Isolation of Natural Polyenes.—(*a*) *From carrot oil*[10,15] (kindly supplied by Nutritional Research Associates Inc., South Whitley, Indiana, U.S.A.). Carrot oil (50 g.) in hexane (100 ml.) was shaken with a solution of potassium hydroxide (40 g.) in methanol (150 ml.) and water (40 ml.) at 40° for 10 hr. and then at 27° for 60 hr. Aqueous potassium hydroxide (60 ml., 35% w/v) and hexane (400 ml.) were added. The mixture was shaken well and set aside for 24 hr., but the resulting emulsion failed to separate. Water (100 ml.) was added, and after 2 days the emulsion had partially separated into 2 layers. The hexane layer was collected and the aqueous layer was rejected. The

EXPERIMENTAL

All operations involving unsaturated compounds were, as far as possible, carried out in an atmosphere of nitrogen and in diffuse light. Solutions of polyenes were evaporated at 30—40° under reduced pressure using a nitrogen bleed.

Light petroleum and benzene were distilled from potassium hydroxide pellets. Ether was boiled under reflux for 3 hr. with either lithium aluminium hydride, or calcium hydride, and then distilled from the excess of reagent. Hexane was freed from aromatic impurities by percolation through a column of silica gel, freshly activated at 200°, (cf. ref. **34**) and then distilled.

Alumina for chromatography was washed as described by Cheeseman *et al.*,[35] and graded.[36]

Melting points were determined in evacuated capillaries and are corrected.

Visible and ultraviolet light absorption data were determined on solutions in hexane, iso-octane, or light petroleum, unless otherwise stated.

[31] P. S. Manchand, U. Schwieter, R. Rüegg, and B. C. L. Weedon, *J. Chem. Soc.*, 1965, 2019.
[32] Private communication.
[33] T. O. M. Nakayama, *Arch. Biochem. Biophys.*, 1958, **75**, 356.
[34] M. M. Graff, R. T. O'Connor, and E. L. Skau, *Ind. Eng. Chem. Anal.*, 1944, **16**, 556.
[35] G. W. H. Cheeseman, Sir Ian Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 1949, 3120.
[36] H. Brockmann and H. Schodder, *Ber.*, 1941, **74**, 73.

residual emulsion was repeatedly extracted with hexane until the extract was colourless. The hexane solutions were combined, washed with 90% aqueous methanol (1 × 200 ml.), and then water (500 ml.) was added. The colourless solid which separated in the hexane layer was re-dissolved by the addition of methanol (150 ml.). Water was added and the mixture was set aside. After 3 days the emulsion had separated. The hexane layer was collected, washed with water (2 × 2 l.), and filtered through sodium sulphate. The hexane solution was concentrated (to 40 ml.), washed with 90% aqueous methanol (3 × 20 ml.) and then with water (3 × 200 ml.). The small amount of emulsion which formed during the water washing was extracted with hexane, and the combined hexane solutions were washed cautiously with water, dried (Na_2SO_4) and evaporated. The residue in ether was added to lithium aluminium hydride (1·0 g.) in ether (200 ml.), and the mixture was boiled under reflux to destroy the lipids present. Further hydride (4·0 g.) in ether (50 ml.) was added. The mixture was boiled under reflux for 1 hr. and then cooled. The excess of hydride was decomposed by the addition of AnalaR acetone (40 ml.). The mixture was evaporated, and the residue was triturated with hexane (200 ml.). The mixture was filtered, and the filtrate was concentrated (to 50 ml.).

Chromatography of the hexane solution on alumina (Grade II), and rechromatography of the appropriate eluates on the same adsorbent using light petroleum (b. p. 60—80°) or hexane as eluant gave many fractions. These included: (i) Phytoene (300 mg.), $\lambda_{max.}$ 297, 286, and 276 mμ, [10^{-3} ε 27·8, 41·2, and 32·4, respectively (lit.,[10] $\lambda_{max.}$ 298, 286, and 275 mμ)], $\nu_{max.}$ (CCl_4) 1665 (ε 25), and 1631 cm.$^{-1}$ (ε 56), $\nu_{max.}$ (liq. film) 1667, 1634, and 766 cm.$^{-1}$. The n.m.r. spectrum (see Figure and Table) was identical with that of a sample (also from carrot oil) supplied by Dr. W. J. Rabourn. (ii) A *cis*-isomer of phytofluene, $\lambda_{max.}$ 367, 347·5, 331·5, 318 (infl.), 304 (infl.), 257 ("*cis*-peak"), and 249 mμ (E_{347}/E_{367} = 1·18). [For "neo B" phytofluene, Rabourn and Quackenbush [13] give $\lambda_{max.}$ 367, 347—8, 331, 318 (infl.), 303 (infl.), and 257 mμ, E_{347}/E_{367} = 1·16]. The n.m.r. spectrum (see Figure and Table) was identical with that of a sample (a mixture of geometrical isomers from carrot oil) supplied by Dr. W. J. Rabourn. (iii) all-*trans*-Phytofluene, $\lambda_{max.}$ 368, 348, and 332 mμ [Rabourn and Quackenbush [13] give $\lambda_{max.}$ 367, 348, 332, and 317 mμ, (10^{-3} ε 85, 88·5, 56, and 27, respectively)]. (iv) A mixture (*ca.* 1 : 1) of all-*trans*- and "neo A" ζ-carotene (*ca.* 10 mg.), $\lambda_{max.}$ 423, 399, 378, 296, and 285 mμ, $\nu_{max.}$ ($CHCl_3$) 1629, 1586, and 968 cm.$^{-1}$ (ε_{968} 495). This sample was used for the n.m.r. studies (see Figure and Table). (v) "neo A" ζ-Carotene (*ca.* 3 mg.), $\lambda_{max.}$ 419, 396, 375, 357 (infl.), 296, and 285 mμ (E_{396}/E_{419} = 2·03; E_{296}/E_{419} = 0·34), (Rabourn and Quackenbush [15] give $\lambda_{max.}$ 420, 396, 375, 358 (infl.), 296, and 286 mμ, E_{396}/E_{420} = 2·04); its n.m.r. spectrum showed no significant difference from that of the mixture of all-*trans*- and "neo A" isomers. (vi) all-*trans*-ζ-Carotene, $\lambda_{max.}$ 424·5, 400·5, 379·5, and 361 (infl.) mμ, no *cis*-peak near 296 mμ [Rabourn and Quackenbush [15] give $\lambda_{max.}$ 426, 401, 380, and 364 (infl.) mμ (10^{-3} ε 138, 137, 84, and 38, respectively)]. When kept in high vacuum for several hours it solidified. Purification from chloroform–methanol gave a yellow solid, m. p. 42—46°, $\lambda_{max.}$ 425, 400, 378, and 361 mμ. It was very unstable in air. Recently, Petzold [37] has claimed to have prepared, from corn endosperm, two solid forms of ζ-carotene, m. p. 28—31° and m. p. 72—74°.

(*b*) *From Chlorella vulgaris* (*mutant G.* 77) (kindly supplied by Dr. M. B. Allen, California). The freeze-dried algæ (50 g.) were powdered and steeped in a mixture of light petroleum (b. p. 30—40°) (120 ml.) and methanol (120 ml.) at 25° for 24 hr. The solid was filtered off, washed with light petroleum (b. p. 30—40°), re-extracted in the same way, and finally extracted with light petroleum (b. p. 30—40°) (2 × 120 ml.; 3 hr. each extraction). The filtrates, extracts, and washings were combined, and an equal volume of water was added. The petroleum layer was separated, dried (Na_2SO_4) and concentrated (to 150 ml.). The residual dark-green solution was added to 20% methanolic potassium hydroxide (200 ml.), and the mixture was kept at 25° for 15 hr. Water was added, and the yellow petroleum layer was separated, washed thoroughly with water, and dried (Na_2SO_4), any emulsions being treated as in the previous isolation. (Spectral analysis indicated the presence of *ca.* 45 mg. of phytoene, traces of phytofluene, and *ca.* 8 mg. of ζ-carotene.) The hydrocarbon extracts were concentrated (to 20 ml.), cooled to 0°, and filtered. Chromatography on alumina (Grade IV), collection of the phytoene and ζ-carotene fractions, destruction of the lipid contaminants with lithium aluminium hydride, and further chromatography gave:—(i) Phytoene (20 mg.), $\lambda_{max.}$ 297, 286, and 276 mμ, $\nu_{max.}$ (CCl_4) 1662 and 1631 cm.$^{-1}$; its n.m.r. spectrum was identical with that of phytoene from carrot oil. (ii) ζ-Carotene isomers containing *ca.* 25% of a pink impurity ($\lambda_{max.}$ *ca.* 448 mμ). The n.m.r. spectrum of the mixture was very similar to that of ζ-carotene from carrot oil in the τ 8—8·5 region, but contained an additional peak near τ 8·7. No bands in the "paraffinic methyl" region were observed.

(*c*) *From Rhodopseudomonas spheroides* (*green mutant*).[33] The natural neurosporene used in the present studies was isolated from this source by Dr. T. O. M. Nakayama, who reported [33] m. p. 117°, $\lambda_{max.}$ 468, 437·5, and 413 mμ, (10^{-3} ε 147, 147, and 95, respectively). The authors are grateful to Dr. Nakayama for making this sample available to them; it has previously been compared with the neurosporene isolated from *Neurospora crassa* by Haxo.[7]

Phytoene Series.—2,6,11,15-*Tetramethylhexadeca*-2,6,8,10,14-*pentaene* (*C_{20}-model*) (XI). Ethereal phenyllithium (3·9 ml., 0·89-N) was added slowly to a stirred suspension of finely-divided geranyl triphenylphosphonium bromide [19] (6·1 g.) in ether. After the mixture had been stirred at 20° for 1 hr., citral (1·9 g.) in ether (10 ml.) was added. The mixture was stirred at 20° for 15 min., heated under reflux for 2 hr., and then cooled. Dilute hydrochloric acid was added, and the product was extracted with ether. The extract was washed with aqueous sodium hydrogen carbonate, dried (Na_2SO_4), and evaporated. Chromatography of the residue in light petroleum (b. p. 60—80°) on alumina (Grade II), and collection and evaporation of the fractions with the required u.v. absorption properties, yielded the C_{20}-*hydrocarbon* (0·52 g.) as a colourless oil; $\lambda_{max.}$ (EtOH) 286 mμ (10^{-3} ε 34), inflexions at 297 and 273 mμ; $\nu_{max.}$ 957 and 766 cm.$^{-1}$ (Found: C, 88·3; H, 11·6. $C_{20}H_{34}$ requires C, 88·15; H, 11·85%).

2,6,10-*Trimethyltrideca*-2,6,8,10-*tetraene* (X). Propyl bromide (8·0 g.) and triphenylphosphine (7·0 g.) were heated at 100° for 15 hr. The product was cooled, ground, and extracted with ether. Crystallisation of the residue from

[37] E. N. Petzold, *Diss. Abs.*, 1959, **20**, 62.

isopropanol gave propyltriphenylphosphonium bromide (9·2 g., 90%), m. p. 236° (Found: C, 65·25; H, 5·9. Calc. for $C_{21}H_{22}BrP$: C, 65·4; H, 5·75%).

ψ-Ionone (2·2 g.) was added slowly to the Wittig reagent prepared from the above salt (4·7 g.) and propyl-lithium in ether (*ca.* 100 ml.). The mixture was stirred at 20° for 30 min., heated under reflux for 2 hr., and then cooled. The solvent was evaporated, and the residue was suspended in 90% aqueous methanol. Isolation of the product with ether, and chromatography as in the previous experiment, gave the *hydrocarbon* (1·2 g., 48%) as a colourless oil, b. p. (bath temp.) 120°/0·8 mm., n_D^{18} 1·5382, $\lambda_{max.}$ (EtOH) 279 mμ (10^{-3} ε 45·6), inflexions at 288 and 269 mμ, $\nu_{max.}$ 1640 and 953 cm.$^{-1}$, τ ($CDCl_3$) 8·25, 8·34, 8·41, and 9·02 (triplet) (Found: C, 87·65; H, 11·9. $C_{16}H_{26}$ requires: C, 88·0; H, 12·0%).

4-*Methyl*-6-(2,6,6-*trimethylcyclohexenyl*)*hexa*-3,5-*diene*. A similar reaction of β-ionone (2·5 g.) to that described above with *ψ*-ionone gave the *hydrocarbon* (1·9 g., 68%) as a colourless oil, b. p. (bath temp.) 80°/0·5 mm., n_D^{22} 1·5183, $\lambda_{max.}$ (EtOH) 247 mμ (10^{-3} ε 13·6), $\nu_{max.}$ 963 cm.$^{-1}$ (Found: C, 87·75; H, 11·8. $C_{16}H_{26}$ requires C, 88·0; H, 12·0%).

3,7,11,15-*Tetramethylhexadeca*-2,6,10,14-*tetraenyl-triphenylphosphonium bromide* (XVI). Phosphorus tribromide (0·83 g.) in light petroleum (b. p. 60—80°) (1 ml.) was added during 1 hr. to a cooled (−7°) solution of *trans*-geranyl-linalool [24] (2·0 g.) and pyridine (0·21 ml.) in the same solvent (3·1 ml.). After the mixture had been stirred at −7° for 3 hr., ice–water was added, and the product was isolated with ether giving geranyl-geranyl bromide (2·39 g.) as a colourless oil, n_D^{21} 1·5060, which was used directly.

A solution of the crude bromide (2·39 g.) and triphenylphosphine (2·1 g.) in ether (50 ml.) was kept at 20° for 2 days. The solid which had separated was collected, washed with ether, and crystallised from ethyl acetate giving the geranyl-geranyl *salt* (2·6 g.), m. p. 127—129° (Found: C, 74·3; H, 7·8. $C_{38}H_{48}BrP$ requires C, 74·1; H, 7·85%).

2,6,10,14,19,23-*Hexamethyltetracosa*-2,6,10,14,16,18,22-*heptaene* (*C_{30}-model*) (XII). Ethereal phenyl-lithium was added slowly to a suspension of the preceding Wittig salt (0·41 g.) in ether (20 ml.) until almost all the solid had reacted. Citral (0·20 g.) in ether (5 ml.) was added to the resulting deep red solution. The mixture was stirred for 30 min. at 20°, heated under reflux for 3 hr., and then cooled and evaporated. Extraction of the product with light petroleum (b. p. 60—80°), and chromatography, gave the *C_{30}-hydrocarbon* (205 mg., 73%), as a colourless oil, $\lambda_{max.}$ (EtOH) 285 mμ (10^{-3} ε 38·8), inflexions at 297 and 273 mμ, $\nu_{max.}$ 953 and 764 cm.$^{-1}$, τ (see Table) (Found: C, 88·1; H, 11·65. $C_{30}H_{48}$ requires C, 88·1; H, 11·85%).

3,7,11,15-*Tetramethylhexadeca*-2,6,10,14-*tetraenal* (*geranyl-citral*) (XVII). 2-Nitropropane (0·84 g.) was added to a solution of potassium hydroxide (0·59 g.) in water (0·95 ml.) and isopropanol (6 ml.). Crude geranyl-geranyl bromide (from 3·9 g. geranyl-linalool) was added, and the mixture was kept at 40—45° for 20 min. Water was added, and the product was extracted with light petroleum (b. p. 40—60°). The extracts were washed, dried (Na_2SO_4), and evaporated giving an oil (1·96 g.), $\lambda_{max.}$ (hexane) 232 mμ (10^{-3} ε 10·7). The crude product was added to a solution of sodium hydrogen sulphite (2·25 g.) in water (4·1 ml.). The mixture was shaken overnight, and then extracted with benzene (the two phases were separated by centrifugation). The semi-solid was suspended in ether at 5°, and 10% aqueous sodium hydroxide was added with stirring. The ethereal layer was separated, washed, dried, and evaporated. Distillation of the residue gave *geranyl-citral* (1·02 g., 35% overall yield), b. p. (bath temp.) 130°/10^{-5} mm., n_D^{20} 1·5031, $\lambda_{max.}$ (hexane) 233 mμ (10^{-3} ε 14·5), $\nu_{max.}$ 1672 cm.$^{-1}$, τ 8·41, 8·34, and 7·83, relative intensities *ca.* 3 : 1 : 1 (Found: C, 83·35; H, 11·0. $C_{20}H_{32}O$ requires C, 83·25; H, 11·20%). Gas–liquid chromatography on poly(ethylene glycol adipate) at 150° indicated the presence of two isomers (presumably *cis*-2 and *trans*-2) in the proportions *ca.* 1 : 10. The 2,4-*dinitrophenylhydrazone* (75% yield) was purified by chromatography from benzene on alumina, and crystallised from methanol, m. p. 58—68°, $\lambda_{max.}$ (EtOH) 386 mμ (10^{-3} ε 19·9) (Found: C, 66·65; H, 7·85; N, 11·9. $C_{26}H_{36}O_4N_4$ requires C, 66·65; H, 7·75; N, 11·95%).

2,6,10,14,19,23,27,31-*Octamethyldotriaconta*-2,6,10,14,16,18,22,26,30-*nonaene* (*Phytoene*) (I). An equivalent of ethereal propyl-lithium was added to a stirred suspension of geranyl-geranyl triphenylphosphonium bromide (XVI) (850 mg.) in ether (50 ml.). The resulting deep red solution was stirred for 30 min. and then geranyl-citral (263 mg.) was added. After the mixture had been boiled under reflux for 2 hr. and cooled, the solvent was evaporated and the residue was suspended in 90% aqueous methanol. Isolation of the product with light petroleum (b. p. 40—60°), and chromatography in the usual manner, gave a mixture of geometrical isomers of phytoene (414 mg., 83%) as a colourless oil, τ (see Table) (Found: C, 88·0; H, 11·6. Calc. for $C_{40}H_{64}$: C, 88·15; H, 11·85%).

The mixture of isomers (360 mg.) in butanol (4 ml.) was added to a saturated solution (8 ml.) of thiourea in butanol, and the solution was kept overnight at 5°. The crystals which had separated were collected, washed thoroughly with light petroleum (b. p. 40—60°), and then shaken with a mixture of water and light petroleum (b. p. 40—60°). The organic layer was separated, washed with water, dried (Na_2SO_4), and evaporated. Chromatography as before gave a mixture (80 mg.) enriched in the all-*trans*-isomer of phytoene, $\lambda_{max.}$ (hexane) 298, 286, and 276 mμ (10^{-3} ε 37·8, 49·8, and 36·6, respectively), $\nu_{max.}$ (cyclohexane) 1665, 1638, 953, and 764 cm.$^{-1}$.

The petroleum filtrate and washings from the above crystalline thiourea complex were combined, washed thoroughly with water, dried ($MgSO_4$), and evaporated. Chromatography as usual gave phytoene as a mixture of isomers (270 mg.), $\lambda_{max.}$ (hexane) 298, 286, and 276 mμ (10^{-3} ε 35·8, 46·7, and 34·6, respectively), $\nu_{max.}$ (cyclohexane) 1665, 1638, 953, and 764 cm.$^{-1}$.

Phytofluene Series.—Farnesal (XX). Farnesyl bromide (9·8 g.) (prepared as described below) was added to a solution of 2-nitropropane (3·36 g.) in isopropanol (24 ml.) and water (3·78 ml.) containing potassium hydroxide (2·37 g.). The mixture was kept at 40—45° for 20 min. and then diluted with water. Isolation and purification of the product as described above for geranyl-citral gave farnesal (4·7 g., 62%), b. p. 94°/0·05 mm., n_D^{18} 1·4980 (lit.,[38] n_D^{20} 1·4980), $\lambda_{max.}$ (hexane) 233 mμ (10^{-3} ε 14).

Methyl 3,7,11,15-*tetramethylhexadeca*-2,4,6,10,14-*pentaenoate* (XXII). 30% Methanolic sodium methoxide (2·2 g.) was added to a mixture of farnesal (0·77 g.), diethyl 3-methoxycarbonyl-2-methylprop-2-enyl-phosphonate (1·75 g.) (for preparation see below), and dimethylformamide (10 ml.) at such a rate that the temperature did not exceed 40°. The mixture was stirred for 1 hr., then neutralised with acetic

[38] Y.-R. Naves, *Helv. Chim. Acta*, 1949, **32**, 1798.

acid, and diluted with water. The mixture was extracted with light petroleum (b. p. 40—60°), and the extracts were washed with aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated. Chromatography of the residue on alumina (Grade IV) from light petroleum, and collection of the main band, gave the *ester* (0·68 g., 62%), b. p. (bath temp.) 140°/2 × 10^{-4} mm., n_D^{15} 1·5572, $\lambda_{max.}$ (EtOH) 318 mμ (10^{-3} ε 33·5), $\nu_{max.}$ 1712 and 1605 $cm.^{-1}$ (Found: C, 79·8; H, 9·95. $C_{21}H_{32}O_2$ requires C, 79·7; H, 10·2%).

3,7,11,15-*Tetramethylhexadeca*-2,4,6,10,14-*pentaen*-1-*ol* (XXIII). The above ester (0·67 g.) in ether (10 ml.) was added to a cooled (−50°) suspension of lithium aluminium hydride (0·17 g.) in ether (25 ml.). The mixture was stirred at −30° for 2·5 hr., and then wet ether was added to decompose the excess of reagent. Saturated aqueous ammonium chloride (0·95 ml.) was added, and the mixture was filtered. The filtrate was washed, dried ($MgSO_4$), and evaporated. Distillation of the residue gave the *alcohol* (0·51 g., 83%) as a colourless oil, n_D^{17} 1·5446, $\lambda_{max.}$ (EtOH) 280 mμ (10^{-3} ε 38·4), $\nu_{max.}$ 3300 $cm.^{-1}$ (Found: C, 82·7; H, 11·1. $C_{20}H_{32}O$ requires C, 83·25; H, 11·2%).

3,7,11,15-*Tetramethylhexadeca*-2,4,6,10,14-*pentaenal* (XXIV). Active manganese dioxide [39] (2·5 g.) was added in portions to a solution of the previously prepared alcohol (XXIII) (0·4 g.) in light petroleum (b. p. 40—60°) (6 ml.). The mixture was shaken for 15 hr. at 20°, and then filtered. The residue was washed thoroughly with ether and the combined filtrate and washings were evaporated. Chromatography of the residue on alumina (Grade IV) from light petroleum (b. p. 40—60°), collection of the main band, and distillation gave the *aldehyde* as a yellow oil, b. p. (bath temp.) 140°/2 × 10^{-4} mm., n_D^{18} 1·5815, $\lambda_{max.}$ (EtOH) 341 mμ (10^{-3} ε 35·2), $\nu_{max.}$ 1661, 1629, and 1597 $cm.^{-1}$. The 2,4-*dinitrophenylhydrazone* (81% yield) was purified by chromatography on alumina (Grade II) using 40% benzene in light petroleum (b. p. 40—60°) as eluant. It crystallised from benzene–light petroleum and had m. p. 112—115°, $\lambda_{max.}$ (EtOH) 419 mμ (10^{-3} ε 43·1) (Found: C, 66·45; H, 7·45; N, 12·2. $C_{26}H_{34}O_4N_4$ requires C, 66·9; H, 7·35; N, 12·0%).

2,6,10,14,19,23,27,31-*Octamethyldotriaconta*-2,6,10,12,14,16,18,22,26,30-*decaene* (*Phytofluene*) (II). An equivalent of ethereal butyl-lithium was added to a suspension of geranyl-geranyl triphenylphosphonium bromide (173 mg.) in ether (25 ml.). The mixture was stirred for 15 min. and then 3,7,11,15-tetramethylhexadeca-2,4,6,10,14-pentaenal (80 mg.) in ether was added. The mixture was stirred for 30 min. and then boiled under reflux for 3 hr. The solvent was evaporated and the residue was suspended in 90% aqueous methanol. The product was extracted with light petroleum (b. p. 40—60°), and the extracts were washed, dried (Na_2SO_4), and evaporated. The residue was purified by repeated chromatography on alumina (Grade IV) using light petroleum (b. p. 40—60°) as eluant. The required bands (located by occasional inspection of the column under u.v. illumination) were collected and evaporated giving the hydrocarbon as a mixture of geometrical isomers (96 mg., 71%). This product in petroleum solution was illuminated in the presence of 2% iodine, and the solution was washed with 2% aqueous sodium thiosulphate, dried, and evaporated. Chromatography of the residue on alumina (Grade II) using light petroleum (b. p. 60—80°) as eluant gave two easily separable bands. Elution of the more strongly adsorbed band with 20% benzene in light petroleum (b. p. 40—60°), and evaporation, gave *trans*-phytofluene as a pale yellow oil with a brilliant green fluorescence; $\lambda_{max.}$ 366, 347, 331, and 318 mμ (10^{-3} ε 82, 85·5, 54, and 26 respectively), $\nu_{max.}$ (CCl_4) 1667, 1631, and 961 $cm.^{-1}$, τ (see Table) (Found: C, 88·5; H, 11·1. Calc. for $C_{40}H_{62}$: C, 88·5; H, 11·5%).

A mixed thin-layer chromatogram of synthetic *trans*-phytofluene, and *trans*-phytofluene extracted from carrot oil, on kieselgel–calcium hydroxide (1 : 4), using light petroleum (b. p. 40—60°) as eluant, revealed no separation. Synthetic phytofluene was easily separated from phytoene and ζ-carotene under these conditions.

ζ-*Carotene Series*.—2,6,10,15,19,23-*Hexamethyltetracosa*-2,6,8,10,12,14,16,18,22-*nonaene* (C_{30}-*model*) (XIII). An equivalent of ethereal phenyl-lithium was added dropwise to a suspension of geranyl triphenylphosphonium bromide [19] (1·5 g.) in ether (30 ml.). The mixture was stirred for 15 min. and then 2,7-dimethyloct-2,4,6-triene-1,8-dial [27] (200 mg.) in methylene dichloride (5 ml.) was added. The mixture was stirred for 30 min. then boiled under reflux for 3 hr., cooled, and evaporated. The residue was suspended in 90% aqueous methanol. Extraction with light petroleum (b. p. 60—80°), chromatography on alumina (Grade IV) using light petroleum (b. p. 60—80°) as eluant, collection of the main band, and evaporation, yielded a yellow solid (345 mg., 74%). Crystallisation from benzene–methanol gave the *hydrocarbon*, m. p. 100—101°, $\lambda_{max.}$ 424, 400, and 379 mμ (10^{-3} ε 133, 131, and 83, respectively), $\nu_{max.}$ ($CHCl_3$) 974 $cm.^{-1}$, τ (see Table) (Found: C, 89·2; H, 11·25. $C_{30}H_{44}$ requires C, 89·0; H, 11·0%).

Farnesyl triphenylphosphonium bromide (XXV). Phosphorus tribromide (1·0 g.) in light petroleum (b. p. 40—60°) (5 ml.) was added during 30 min. to a solution of *trans*-nerolidol (1·0 g.) in the same solvent (7 ml.) at −7°, and the mixture was stirred for 2·5 hr. Ice–water was added, and the product was extracted with ether. The extracts were washed with aqueous sodium hydrogen carbonate, dried (Na_2SO_4), and evaporated, giving farnesyl bromide (1·04 g.) as a colourless oil, n_D^{22} 1·5070. The crude bromide was added to a solution of triphenylphosphine (1·2 g.) in ether (25 ml.) and the mixture was kept for 2 days. The solid which had separated was collected and extracted with boiling benzene. Crystallisation of the residue from ethyl acetate gave the *salt*, (1·3 g., 53%), m. p. 128—129° (Found: C, 72·1; H, 7·0. $C_{33}H_{40}PBr$ requires C, 72·2; H, 7·35%).

2,6,10,14,19,23,27,31-*Octamethyldotriacont*-2,6,10,12,14,16,18,20,22,26,30-*undecaene* (ζ-*carotene*) (III*a*). An equivalent of ethereal propyl-lithium was added to a suspension of farnesyl triphenylphosphonium bromide (252 mg.) in ether (20 ml.), and the mixture was stirred for 15 min. A solution of 2,7-dimethylocta-2,4,6-triene-1,8-dial [27] (35 mg.) in methylene dichloride (3 ml.) was added, and the mixture was boiled under reflux for 3 hr., then cooled and evaporated. The residue was suspended in 90% aqueous methanol, and the product was extracted with light petroleum (b. p. 40—60°). Chromatography on alumina (Grade II), using light petroleum–benzene mixtures as eluant, gave a yellow oil (102 mg., 85%) which consisted of geometrical isomers of the required hydrocarbon. Further chromatography of this mixture on alumina (Grade II) using a mixture of benzene and light petroleum (1 : 4) as eluant, collection of the appropriate band, and evaporation, yielded the all-*trans*-isomer which separated

[39] J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jensen, and T. Walker, *J. Chem. Soc.*, 1952, 1094.

from chloroform–methanol and had m. p. 38—42° (undepressed on admixture with the natural pigment), $\lambda_{max.}$ 425, 401, 380, and 360 mμ (10^{-3} ε 138, 138, 90, and 40·6, respectively), $\nu_{max.}$ (C_6H_{12}) 966 cm.$^{-1}$, τ (see Table) (Found: C, 88·6; H, 11·2. Calc. for $C_{40}H_{60}$: C, 88·8; H, 11·2%).

Natural and synthetic all-*trans*-ζ-carotene did not separate during mixed chromatograms on columns of alumina (Grade II) using 10% benzene in light petroleum (b. p. 60—80°) as eluant, or on thin layers of kieselgel–calcium hydroxide (1 : 4) using light petroleum (b. p. 60—80°) as eluant.

Both natural and synthetic pigments exhibited the same behaviour on iodine catalysed stereomutation. A solution of all-*trans*-ζ-carotene in light petroleum (b. p. 40—60°) containing iodine (2% based on ζ-carotene) was kept for 10 min. at a distance of 1 ft. from a 60 w tungsten filament lamp. The solution was then washed with 2% aqueous sodium thiosulphate, dried and evaporated. Chromatography of the residue on alumina (Grade II) using 10% benzene in light petroleum (b. p. 40—60°) as eluant, gave two bands. The less strongly adsorbed compound had $\lambda_{max.}$ 419, 395, 374, 296, and 286 mμ (10^{-3} ε 86·4, 105, 75, 20·8, and 16, respectively, on the *cis*-isomer obtained from synthetic ζ-carotene), $\nu_{max.}$ (cyclohexane) 950 cm.$^{-1}$. Stereomutation of this component gave a mixture which exhibited more intense absorption with maxima at 424, 400, and 379 mμ. The second, and major, component of the stereomutation mixture was eluted with 30% benzene in light petroleum (b. p. 40—60°) and had $\lambda_{max.}$ 425, 401, and 379 mμ, corresponding to the all-*trans*-isomer. Further stereomutation of this component resulted in a marked reduction in intensity of absorption, and a slight shift of $\lambda_{max.}$ to shorter wavelengths.

2,6,10,14,19,23,27,31-*Octamethyldotriaconta*-2,6,10,12,14,18,20,22,26,30-*decaen*-16-*yne* (15,15′-*Dehydro*-ζ-*carotene*) (XXVIII). Ethereal butyl-lithium was added to a stirred suspension of farnesyl triphenylphosphonium bromide (0·499 g.) in dry ether, and then 2,7-dimethylocta-2,6-dien-4-yne-1,8-dial [27] (58·3 mg.) in chloroform (5 ml.) was added. The mixture was stirred at 20° for 30 min., then boiled under reflux for 2 hr., cooled, and evaporated. Extraction of the product in the usual way and chromatography on alumina (Grade IV) using light petroleum (b. p. 60—80°) as eluant, collection of the main band, and evaporation, gave a yellow crystalline solid (170 mg., 82%). Further chromatography on alumina (Grade II) separated this product into three geometrical isomers. The first was only present in trace amounts, but the second isomer accounted for about one third of the total hydrocarbon. Both of these (*cis*-) isomers had $\lambda_{max.}$ 409 and 387 mμ, the latter band being the more intense. Elution of the third isomer, and crystallisation from chloroform–methanol gave *all*-trans-*dehydro*-ζ-*carotene*, m. p. 71—72°, $\lambda_{max.}$ 409, 387, and 366 (infl.) mμ (10^{-3} ε 82·5 and 82·5), τ ($CDCl_3$) 7·92, 8·21 8·32, and 3·39, relative intensities *ca.* 1 : 1 : 1 : 2 (Found: C, 89·5; H, 10·75. $C_{40}H_{58}$ requires C, 89·15; H, 10·85%).

cis-15-ζ-*Carotene* (with P. S. Manchand). *trans*-Dehydro-ζ-carotene (38·2 mg.) in ethyl acetate (5 ml.) was shaken in hydrogen with Lindlar catalyst [28] (20 mg.) until 1·1 moles had been absorbed. The mixture was filtered, the filtrate was evaporated to dryness at 10° in total darkness, and the residue was used immediately for the measurement of spectroscopic constants, $\lambda_{max.}$ 422, 398, 378, 296, and 286 mμ (10^{-3} ε 41·3, 58·4, 50·3, 20·8, and 18·6), respectively, $\nu_{max.}$ (C_6H_{12}) 957 and 777 cm.$^{-1}$.

3-*Methoxycarbonyl*-2-*methylprop*-2-*enyl triphenylphosphonium bromide*. A solution of methyl 4-bromosenecioate [40] (5·52 g.) (prepared from methyl senecioate in 75% yield) and triphenylphosphine (8·3 g.) in ether (100 ml.) was boiled under reflux for 10 hr. and then cooled. The solid which had separated was filtered off and washed with ether. Crystallisation from isopropanol gave the *salt* (10·5 g., 81%), m. p. 179° (Found: C, 62·95; H, 5·25. $C_{24}H_{24}BrO_2P$ requires: C, 63·3; H, 5·3%).

Diethyl 3-*methoxycarbonyl*-2-*methylprop*-2-*enylphosphonate* (XXI). Methyl 4-bromosenecioate [40] (5·0 g.) was slowly added to triethylphosphite (6·0 g.) at 100°. The temperature of the mixture was slowly raised to 150° and was maintained at 150° for 30 min. Distillation of the mixture then gave the *phosphonate* (5·14 g., 79%), b. p. 120—122°/0·6 mm., n_D^{19} 1·4605 (Found: C, 47·7; H, 7·45. $C_{10}H_{19}O_5P$ requires C, 48·0; H, 7·65%). Gas–liquid chromatography and n.m.r. spectroscopy indicated the presence of *cis*- and *trans*-isomers in the proportion of 2 : 3.

Methyl 3,7,11-*trimethyldodeca*-2,4,6,10-*tetraenoate* (XXX). (*a*) Commercial ψ-ionone was distilled and the fraction b. p. 129—133°/4·5 mm. was collected. Gas–liquid chromatography on Apiezon T at 150° showed this to be a mixture of two isomers in the ratio 5 : 4.

Methoxyacetylene [41] (3·1 g.) in dry ether (10 ml.) was added to a stirred solution of ethylmagnesium bromide (from 1·4 g. of magnesium) in ether at −70°. The mixture was allowed to warm to 20° and was then heated under reflux for 2 hr. (solid carbon dioxide condenser). The resulting suspension was cooled to 0°, and ψ-ionone (5·0 g.) in ether (10 ml.) was added. The mixture was stirred and heated under reflux for 13 hr. and then cooled to 0°. Saturated aqueous ammonium chloride (from 13 g. of NH_4Cl) was added, and the mixture was extracted with ether. The extracts were washed, dried (Na_2SO_4), and evaporated to give the crude acetylenic alcohol which was used without purification, $\nu_{max.}$ 3333 and 2252 cm.$^{-1}$.

10% Aqueous sulphuric acid (3 ml.) was added to the crude product in methanol (25 ml.). The mixture gently heated under reflux after the addition and was kept for 2 hr. Water was added and the product was extracted with ether. The extracts were washed with aqueous sodium hydrogen carbonate, dried (Na_2SO_4), and evaporated. Chromatography of the residual brown oil on alumina (Grade IV) using light petroleum (b. p. 60—80°) as eluant, collection of the main band, and distillation, gave the *ester* (3·16 g., 49%) as a pale yellow oil, b. p. (bath temp.) 100°/10^{-4} mm., n_D^{19} 1·5565, $\lambda_{max.}$ (EtOH) 314 mμ (10^{-3} ε 30·5) (Found: C, 77·0; H, 9·8. $C_{16}H_{24}O_2$ requires C, 77·35; H, 9·75%). Gas–liquid chromatography on Apiezon T at 150° showed that this compound was a mixture of four geometrical isomers in comparable proportions.

(*b*) An equivalent of aqueous sodium hydroxide was added with stirring to a solution of 3-methoxycarbonyl-2-methylpropenyl triphenylphosphonium bromide (4·0 g.) in water (10 ml.). The yellow precipitate (2·3 g.) of phosphoran was filtered off, washed with water, and dried *in vacuo*. A mixture of the phosphoran (2·3 g.), citral

[40] K. Ziegler, A. Späth, E. Schaaf, W. Schumann, and E. Winkelmann, *Annalen*, 1942, **551**, 80.

[41] G. Eglinton, E. R. H. Jones, B. L. Shaw, and M. C. Whiting, *J. Chem. Soc.*, 1954, 1860.

(0·9 g.), and benzene (20 ml.) was boiled under reflux for 14 hr., then cooled and evaporated. Extraction of the residue with light petroleum (b. p. 40—60°), and chromatography of the extracts on alumina (Grade II), yielded the ester (0·69 g., 47%) as a yellow oil, b. p. (bath temp.) 115°/10^{-4} mm., $\lambda_{max.}$ (EtOH) 314 mμ (10^{-3} ε 30·8), $\nu_{max.}$ 1709 and 1600 $cm.^{-1}$.

(*c*) 30% Methanolic sodium methoxide (39 g.) in dimethylformamide (30 ml.) was added to a mixture of diethyl 3-methoxycarbonyl-2-methylpropenylphosphonate (32·4 g.), redistilled citral (15·1 g.) and dimethylformamide (100 ml.) at such a rate that the temperature did not exceed 40°. The mixture was stirred for a further 45 min. and then glacial acetic acid was added (to neutralise the excess of base), followed by water. The mixture was extracted with light petroleum (b. p. 40—60°), and the extracts were washed, dried, and evaporated. The residual oil was distilled giving the ester (16·5 g., 67%), b. p. 105—110°/2 × 10^{-4} mm., n_D^{13} 1·5686, $\lambda_{max.}$ (EtOH) 314 mμ (10^{-3} ε 31·1), $\nu_{max.}$ 1704 and 1603 $cm.^{-1}$. Gas–liquid chromatography on poly(ethylene glycol adipate) at 150° gave two bands in the ratio 2 : 3.

3,7,11-*Trimethyldodeca*-2,4,6,10-*tetraen*-1-*ol* (XXXII). (*a*) A solution of the preceding ester (XXX) (7·96 g.) in ether (20 ml.) was added slowly to a cooled (−50°) suspension of lithium aluminium hydride (1·8 g.) in ether (50 ml.). The temperature was allowed to rise to 0° and the mixture was stirred at 0° for 1 hr. Wet ether was added to decompose the excess of reagent, and then saturated aqueous ammonium chloride (10 ml.). The mixture was filtered and the filtrate was washed, dried ($MgSO_4$), and evaporated. Distillation of the residual oil gave the *alcohol* (6·52 g., 92%) as a colourless oil, b. p. 100—105°/10^{-4} mm., n_D^{18} 1·5561, $\lambda_{max.}$ (EtOH) 281 mμ (10^{-3} ε 35·3), $\nu_{max.}$ 3170 and 952 $cm.^{-1}$ (Found: C, 81·75; H, 10·95. $C_{15}H_{24}O$ requires C, 81·75; H, 11·0%).

(*b*) [42] A solution of potassamide in liquid ammonia (*ca.* 1 l.) was prepared from potassium (9·5 g.). A mixture of citral (15 g.) and methyl senecioate (15·6 g.) in dry ether (75 ml.) was added, and the mixture was stirred for 3 days. The ammonia was then allowed to evaporate, and a mixture of methanol (50 ml.), water (15 ml.), and potassium hydroxide (2 g.) was added. The resulting mixture was boiled under reflux for 2 hr. and then the methanol was distilled off. The residue was acidified with 10% hydrochloric acid, extracted with ether, and the ethereal solutions were extracted with aqueous sodium hydrogen carbonate. These alkaline extracts were acidified, and the crude acidic product was isolated with ether as a brown oil (25 g.), $\lambda_{max.}$ (EtOH) 311 mμ, E_1^1 = 470. The crude product in ether (50 ml.) was added slowly to a stirred suspension of lithium aluminium hydride (3·5 g.) in ether (200 ml.) at −30°. The mixture was stirred at 0° for 1 hr. and the product was isolated in the usual way. Distillation yielded the crude alcohol (4·1 g., 19%) as a pale yellow oil, $\lambda_{max.}$ (EtOH) 281 mμ (E_1^1 = 1170).

3,7,11-*Trimethyldodeca*-2,4,6,10-*tetraenal* (XXXIII). Manganese dioxide (71 g.), prepared by the method of Attenburrow,[29] was added in portions to a solution of the preceding alcohol (XXXII) (12·6 g.), from method (*a*), in light petroleum (b. p. 40—60°) (100 ml.) at such a rate that the temperature did not exceed 30°. The mixture was shaken for 15 hr. and then filtered, and the residue was washed throughly with ether. The filtrate and washings were evaporated, and the residual oil was distilled to give the *aldehyde* (11·1 g., 88%) as a yellow oil, b. p. 98—100°/10^{-4} mm., n_D^{15} 1·6061, $\lambda_{max.}$ (EtOH) 341 mμ (ε × 10^{-3} = 36·6), $\nu_{max.}$ 1661, 1597, and 961 $cm.^{-1}$ (Found: C, 82·6; H, 10·15. $C_{15}H_{22}O$ requires C, 82·5; H, 10·15%). The 2,4-*dinitrophenylhydrazone* (prepared in 85% yield) was purified by chromatography on alumina (Grade II) using benzene as eluant. It crystallised from methanol and had m. p. 105—115°, $\lambda_{max.}$ (EtOH) 418 mμ (10^{-3} ε 42·6) (Found: C, 63·35; H, 6·6; N, 14·05. $C_{21}H_{26}N_4O_4$ requires C, 63·3; H, 6·6; N, 14·05%).

Methyl 3,7,11,15-*tetramethylhexadeca*-2,4,6,8,10,14-*hexaenoate* (XXXIV). 30% Methanolic sodium methoxide (4·1 g.) was added to a mixture of the preceding aldehyde (XXXIII) (1·98 g.) and diethyl 3-methoxycarbonyl-2-methylprop-2-enylphosphonate in dimethylformamide (25 ml.) at such a rate that the temperature did not exceed 40°. The mixture was stirred for 1 hr., then neutralised with glacial acetic acid and diluted with water. The mixture was extracted with light petroleum (b. p. 40—60°), the extracts were washed, dried ($MgSO_4$), and evaporated. Distillation of the residual oil gave the *ester* (1·6 g., 55%) as an orange oil, b. p. (bath temp.) 135°/10^{-5} mm., $\lambda_{max.}$ (EtOH) 370 mμ (10^{-3} ε 48·1), $\nu_{max.}$ 1712 $cm.^{-1}$ (Found: C, 79·75; H, 9·5. $C_{21}H_{30}O_2$ requires C, 80·2; H, 9·6%). (The refractive index could not be determined with an Abbé refractometer.)

3,7,11,15-*Tetramethylhexadeca*-2,4,6,8,10,14-*hexaen*-1-*ol* ("γ-*Vitamin A*") (XXXV). A solution of the preceding ester (XXXIV) (5·2 g.) in dry ether (20 ml.) was added slowly to a suspension of lithium aluminium hydride (0·7 g.) in ether (50 ml.) at −60°. The mixture was allowed to warm to −30° and kept at −30° for 2 hr. Wet ether was added (to decompose the excess of reagent), followed by saturated aqueous ammonium chloride (3·9 ml.). The mixture was filtered, and the filtrate was washed and dried ($MgSO_4$). Chromatography of the residual oil on alumina (Grade IV) using benzene–light petroleum mixtures as eluant, isolation of the main fraction, and distillation in high vacuum gave the alcohol (3·5 g., 74%) as a mixture of isomers which crystallised and had m. p. (Kofler hot-stage apparatus) 64—67°, $\lambda_{max.}$ (EtOH) 360, 343, and 326 mμ (E_1^1 = 1520, 1740, and 1300, respectively) $\nu_{max.}$ 3330, and 956 $cm.^{-1}$ [lit.,[31] m. p. 107—108°, $\lambda_{max.}$ (EtOH) 359, 341, and 324 mμ (10^{-3} ε 78·5, 86, and 56, respectively)].

3,7,11,15-*Tetramethylhexadeca*-2,4,6,8,10,14-*hexaenal* ("γ-Retinal") (XXXVI). Active manganese dioxide [39] (16·5 g.) was added in portions to a solution of the preceding alcohol (XXXV) (3·3 g.) in light petroleum (b. p. 40—60°), the temperature being kept below 30°. The mixture was shaken at 20° for 12 hr. and then filtered. The residue was thoroughly washed with ether, and the combined filtrate and washing were evaporated. Chromatography of the residual oil on alumina (Grade IV) using light petroleum (b. p. 40—60°) as eluant, isolation of the main fraction, and distillation, gave the aldehyde (2·52 g., 76%) as a mixture of isomers, b. p. (bath temp.) 135°/10^{-5} mm., $\lambda_{max.}$ (EtOH) 400 mμ (E_1^1 = 2000), $\nu_{max.}$ 1658 and 961 $cm.^{-1}$ (Found: C, 84·15; H, 9·5. Calc. for $C_{20}H_{28}O$: C, 84·45; H, 9·9%) [lit.,[31] m. p. 67—68°, $\lambda_{max.}$ (EtOH) 408 mμ (10^{-3} ε = 57)].

2,6,10,14,19,23,27,31-*Octamethyldotriaconta*-2,6,8,10,12,14,16,18,22,26,30-*undecaene* (III*b*). An equi-

[42] Cf. M. Matsui, K. Yamashita, M. Miyano, S. Kitamura, S. Okano, A. Koboyashi, T. Sato, and R. Mikami, *Proc. Japan Acad.*, 1958, **34**, 220; M. Matsui, S. Okano, K. Yamashita, M. Miyano, S. Kitamura, A. Koboyashi, T. Sato, and R. Mikami, *J. Vitaminol.*, 1958, **4**, 178.

valent of ethereal propyl-lithium was added to a suspension of geranyl-geranyl triphenylphosphonium bromide (500 mg.) in ether (20 ml.). The mixture was stirred for 15 min. and then "γ-retinal" (143 mg.) in methylene dichloride (2 ml.) was added. The mixture was boiled under reflux for 2 hr. then cooled and evaporated. The residue was suspended in 90% aqueous methanol, and the product was extracted with light petroleum (b. p. 40—60°). The extracts were washed, dried ($MgSO_4$) and concentrated, and then subjected to chromatography on alumina (Grade II) using light petroleum–benzene mixtures as eluant. Collection of the main band and evaporation gave the hydrocarbon as a yellow oil. This product was illuminated in petroleum solution in the presence of 2% iodine. The solution was washed with 2% aqueous sodium thiosulphate, dried, and concentrated. Chromatography on alumina (Grade II) using light petroleum (b. p. 40—60°)–benzene as eluant, collection and evaporation of the main band, gave the all-*trans*-isomer as a semi-solid, $\lambda_{max.}$ 420, 395, 374, and 356 (shoulder) mμ (10^{-3} ε 136, 136, 83, and 39 respectively), $\nu_{max.}$ (cyclohexane) 1634 and 955 $cm.^{-1}$, $\nu_{max.}$ (CCl_4) 1629 and 961 $cm.^{-1}$.

Neurosporene Series.—2,6,10,15,19,23-*Hexamethyltetracosa*-4,6,8,10,12,14,16,18,20-*nonaene* (VII*b*). A mixture of isopentyl bromide (7·5 g.) and triphenylphosphine (13·1 g.) in benzene (60 ml.) was boiled under reflux for 60 hr. and then cooled. The crystals (14·3 g.) which had separated were collected and dried, and had m. p. 160—161°.

An equivalent of ethereal propyl-lithium was added to a suspension of the crude Wittig salt (1·0 g.) in dry tetrahydrofuran (20 ml.). The mixture was stirred for 30 min. and then crocetindial [19] (100 mg.) in methylene dichloride (5 ml.) was added. The mixture was stirred for 15 min., boiled under reflux for 1 hr., cooled, and evaporated. The residue was suspended in 90% aqueous methanol and the product was extracted with light petroleum (b. p. 40—60°). The extracts were washed, dried ($MgSO_4$), and concentrated. Chromatography on alumina (Grade II) using light petroleum (b. p. 60—80°) as eluant, collection of the main band, evaporation, and crystallisation from benzene–methanol, gave the *hydrocarbon* (105 mg., 77%), m. p. 167° (corr.), $\lambda_{max.}$ 465, 435, and 414 mμ (10^{-3} ε 146, 145, and 84 respectively), τ (see Table) (Found: C, 88·55; H, 10·85. $C_{30}H_{44}$ requires C, 89·0; H, 10·95%).

2,6,10,14,19,23,27,31-*Octamethyldotriaconta*-2,6,8,10,12,14,16,18,20,22,26,30-*dodecaene* (*Neurosporene*) (IV). (*a*) Phosphorus tribromide (0·12 g.) in light petroleum (b. p. 40—60°) (3 ml.) was added during 10 min. to a solution of 3,7,11,15-tetramethylhexadeca-2,4,6,10,14-pentaen-1-ol (234 mg.) in the same solvent at −50°. The mixture was stirred for 10 min. at −30°, and then ice–water was added. The mixture was extracted with ether, the extracts were washed with aqueous hydrogen carbonate, dried ($MgSO_4$), and concentrated, and triphenylphosphine (0·4 g.) was added. The mixture was shaken until a clear solution was obtained, and then it was evaporated nearly to dryness and left over-night. The mixture was dried *in vacuo* at 40° for 10 min. and then extracted with boiling ether and again dried *in vacuo*. The product was a brown resinous oil.

An equivalent of ethereal butyl-lithium was added to this product, covered with dry ether (20 ml.), and the mixture was stirred for 30 min. "γ-Retinal" (200 mg.) in methylene dichloride (5 ml.) was added, and the mixture was boiled under reflux for 3 hr. and then cooled. The solvent was evaporated and the residue was suspended in 90% aqueous methanol. The product was extracted with light petroleum (b. p. 40—60°), and the extracts were washed, dried, and evaporated. Chromatography of the residue on alumina (Grade III) using light petroleum (b. p. 40—60°)–benzene as eluant, collection of the main band, and evaporation, gave neurosporene (42 mg.) as a red semi-solid. The product, in light petroleum, was illuminated with a 60 w tungsten filament lamp for 10 min. in the presence of iodine (2%). The solution was washed with 2% aqueous sodium thiosulphate, dried, and concentrated. Chromatography on alumina (Grade II) using light petroleum–benzene mixtures as eluant, collection of the main band, evaporation, and repeated crystallisation from ethanol, gave all-*trans*-neurosporene, m. p. 115—116° (corr.) (undepressed on admixture with a sample of natural neurosporene supplied by Dr. T. Nakayama), $\lambda_{max.}$ 470, 440, 416, and 398 mμ (10^{-3} ε 155, 155, 100, and 57·6, respectively), $\nu_{max.}$ (CCl_4) 1631 and 961 $cm.^{-1}$, τ (see Table).

(*b*) An equivalent of ethereal propyl-lithium was added to a suspension of farnesyl triphenylphosphonium bromide (155 mg.) in ether. A solution of (C_{25}-) apo-12′-lycopenal [31] (50 mg.) in methylene dichloride (5 ml.) was added, and the mixture was stirred for 30 min. at 20°, and then boiled under reflux for 2 hr. Isolation of the product in the usual way, and chromatography on alumina (Grade II) using light petroleum–benzene mixtures as eluant, gave a red solid (59 mg., 76%). Repeated crystallisation from ethanol gave all-*trans*-neurosporene, m. p. 116°, $\lambda_{max.}$ 470, 440, 415, and 398 mμ (10^{-3} ε 157, 157, 86·9, and 55·1, respectively), $\nu_{max.}$ (CCl_4) 1630, and 960 $cm.^{-1}$. A mixed chromatoplate with natural neurosporene, using calcium hydroxide–kieselgel as stationary phase, and light petroleum (b. p. 60—80°) as eluant, resulted in no separation of the two pigments.

Frequent reference has been made throughout this Paper to the help, and information in advance of general publication, provided by other workers and various organisations. It is also a pleasure to thank the Royal Society for a research grant (to B. C. L. W.), the D.S.I.R. for a Research Studentship (to P. T. S.), and Roche Products Ltd. (Welwyn Garden City) and Hoffmann–La Roche Ltd. (Basle) for financial assistance and generous gifts of chemicals.

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