Application of Nuclear Magnetic Resonance Spectroscopy in the Stereochemical Assignment of Poly-Z-Isomeric Conjugated Polyene Isoprenoids

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[13]C N.m.r. spectra of a range of synthetic poly-Z-polyene isoprenoids, *i.e.* Z-isomers of the tetraenoate (1), the hexaenal (3), and the nonaene (4), are presented and discussed. Carbon-13 magnetic resonance isomerisation shift data for the vinyl methyl and vinyl methylene carbon atoms in Z-E-isomers of (1), (3), and (4) are shown to provide a general protocol for the unambiguous assignment of geometry amongst polyene isoprenoids.

[1]H N.m.r. shift data obtained for the vinyl methyl and vinyl methylene groups in isomers of the polyenes (1), (3), and (4) are also discussed and shown to be less useful in determining the structures of poly-Z-polyene isoprenoids.

The majority of naturally occurring carotenoids show an all-E-configuration about their polyene chromophores. Some of the most intriguing members of this class of natural product, however, have one or more Z-double bonds.[1,2] Amongst these members, the biosynthetically significant poly-Z-carotenoid pigments, *e.g.* ' prolycopene ' and ' proneurosporene ', present in fruits of the Tangerine tomato, are perhaps the most intriguing.[2]

Although a number of spectroscopic investigations of the structures of poly-Z-carotenoids have been made, early studies were hampered not only by the lack of reliable spectroscopic techniques but also by the dearth of suitable synthetic model compounds on which to base and define accurate spectroscopic parameters.[3] With the introduction of highfield [1]H n.m.r. and [13]C n.m.r. spectroscopy in recent years, the first of these stumbling-blocks has been largely removed.[4]

In connection with our structural investigations of the poly-Z-carotenoids and their congeners present in Tangerine tomato fruits,[5] it became necessary (a) to develop unambiguous syntheses of a range of model poly-Z-polyene isoprenoids, and (b) to derive reliable E-Z-isomerisation n.m.r. shift parameters from these model compounds such that the number and position of any Z-double bond set within an unknown carotenoid polyene system could be pin-pointed with accuracy. In previous publications we have summarised our efforts to achieve satisfactory and unambiguous syntheses of a range of poly-Z-polyene isoprenoids.[6] In this paper we show how we have used the n.m.r. spectra of these model compounds to derive [13]C n.m.r. and [1]H n.m.r. E-Z-isomerisation shift parameters which are useful in assigning the stereochemistry of almost any poly-Z-polyene isoprenoid and carotenoid. In the accompanying paper[7] we describe how these n.m.r. shift parameters were used to assign stereochemistries to the poly-Z-carotenoids found in fruits of the Tangerine tomato.

[13]C N.m.r. Spectroscopy.—[13]C N.m.r. spectroscopy has a number of advantages over all previously used spectroscopic techniques for assigning accurately the stereochemistry of Z-E-carotenoids. Not least, the noise-decoupled [13]C n.m.r. spectrum of a carotenoid shows immediately whether or not the sample is homogeneous—a feature not always apparent with other spectroscopic techniques. In addition, the number of signals in the noise-decoupled spectrum instantly reveals whether the Z-E-carotenoid has an isomerically symmetrical polyene chromophore.

Although almost every carbon atom in a polyene isoprenoid is sensitive to changes in stereochemistry,[4] except in the most simple instances, the olefinic carbon atoms in the [13]C n.m.r.

spectra of these molecules are usually difficult to assign with accuracy. We have found that it is relatively easy to assign the vinyl methyl and the vinyl methylene carbon atoms in a range of authentic Z-E-polyene isoprenoids, and that [13]C n.m.r. shift parameters on these resonances alone can be used to assign Z-E-stereochemistry within this class of compound.
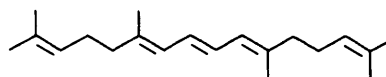
Our previous synthetic investigations had made available all possible geometrical isomers of the tetraenoate (1) and the pentaene (2), and selected Z-E-isomers of the hexaenal (3) and of the nonaene (4). [13]C N.m.r. shifts for the vinyl methyl and vinyl methylene carbon atoms in Z-E-isomers of compounds (1), (3) and (4) are collected on formulae (5)—(19). The assignments followed from noise decoupled spectra and from correlation and comparison of shift data with those of Z-E-isomers of vitamin A,[8] capsorubin,[9] zeaxanthin[9] and the pentaene (2).[6a] Using the data on formulae (5)—(19), the [13]C n.m.r. isomerisation shifts $\Delta = \delta_Z - \delta_E$ shown in Figure 1 were derived. The shifts are given as differences in p.p.m. from the corresponding all-E-polyene segment, with a positive sign representing a deshielding effect.

The [13]C n.m.r. shifts of vinyl methyl and vinyl methylene carbon atoms associated with isolated trisubstituted double bonds are critically dependent on the configuration of the double bond as a result of the well-known γ-effect.[10] The data summarised on formulae (20) and (21) for the 2E,6E- and 2E,6Z-isomers respectively of farnesol illustrate clearly this effect. Inspection of [13]C n.m.r. shift data for the vinyl methyl and vinyl methylene carbon atoms at the end of the polyene chromophores in isomers (5)—(19) demonstrates that the shifts are not only dependent on the configuration of the double bond to which they are attached, but also on the configuration of the double bonds (*i.e.* C-7, C-8) with which they are conjugated [see formulae (22)—(24)]. A change in configuration of the C-9, C-10 double bond induces a change in the 5-Me shift of no more than 0.1 p.p.m., a value which is close to the limits of instrument accuracy. Referring to structure (29), it is clear then that the shift data for 4- and 5-Me indicate not only the geometry of the C-5, C-6 double bond but also the geometry of the conjugated double bond at C-7, C-8.
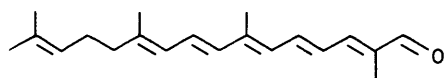
The [13]C n.m.r. shift data derived for the vinyl methyl carbon atom at C-9 [structure (29)], and summarised in Figure 1 [formulae (25)—(28)], provide an extremely reliable probe of the stereochemistries of the three neighbouring bonds at C-7, C-8; C-9, C-10; and C-11, C-12. The shift data are average values from figures recorded for the geometrical isomers of the model compounds (1), (3), and (4), and also from published figures for isomers of vitamin A,[8] capsorubin,[9] and zeaxanthin.[9] The data shown on formulae (25)—(28)
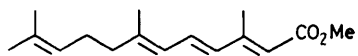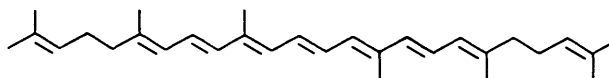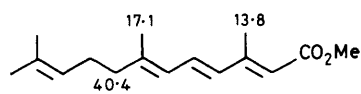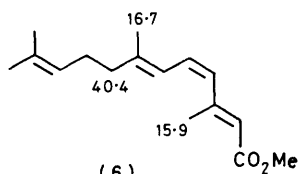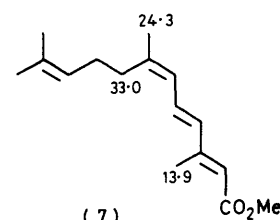
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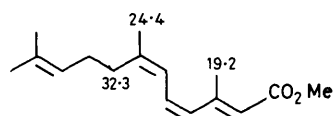
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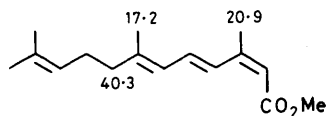
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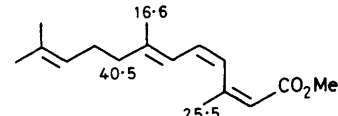
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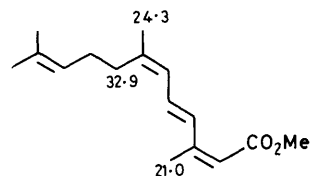
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+ 7·2

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+ 7·5

−8·1

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− 0·4

+ 0·1

(24)

+ 4·4

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+ 7·8

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− 0·3

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+ 11·5

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4 6 8 10 12 14

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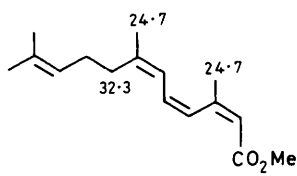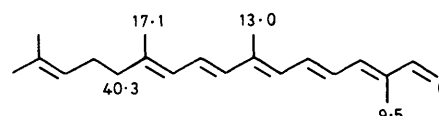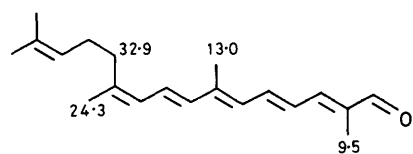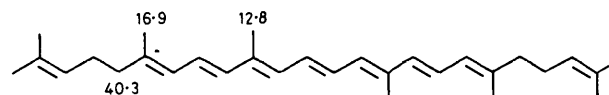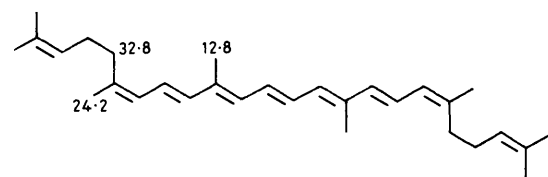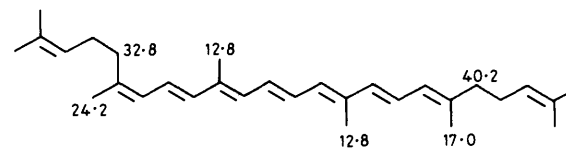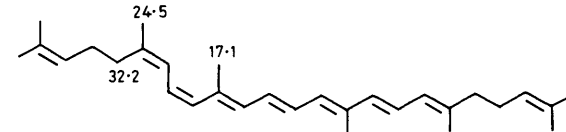
Figure 1. Average values of the ^{13}C n.m.r. isomerisation shifts $\Delta = \delta_Z - \delta_E$ in p.p.m. (CDCl$_3$) for vinyl methyl and vinyl methylene carbon atoms on mono- and di-Z-polyene polyisoprenoids

demonstrate that an inversion of stereochemistry at either C-7, C-8 or C-9, C-10 has a dramatic effect on the chemical shift of the 9-Me carbon resonance. By contrast, an $E \long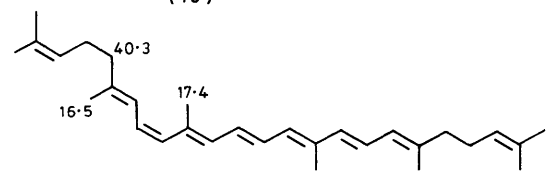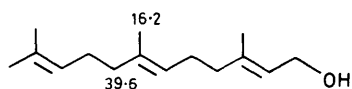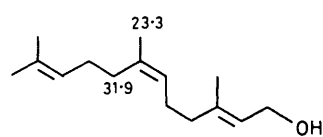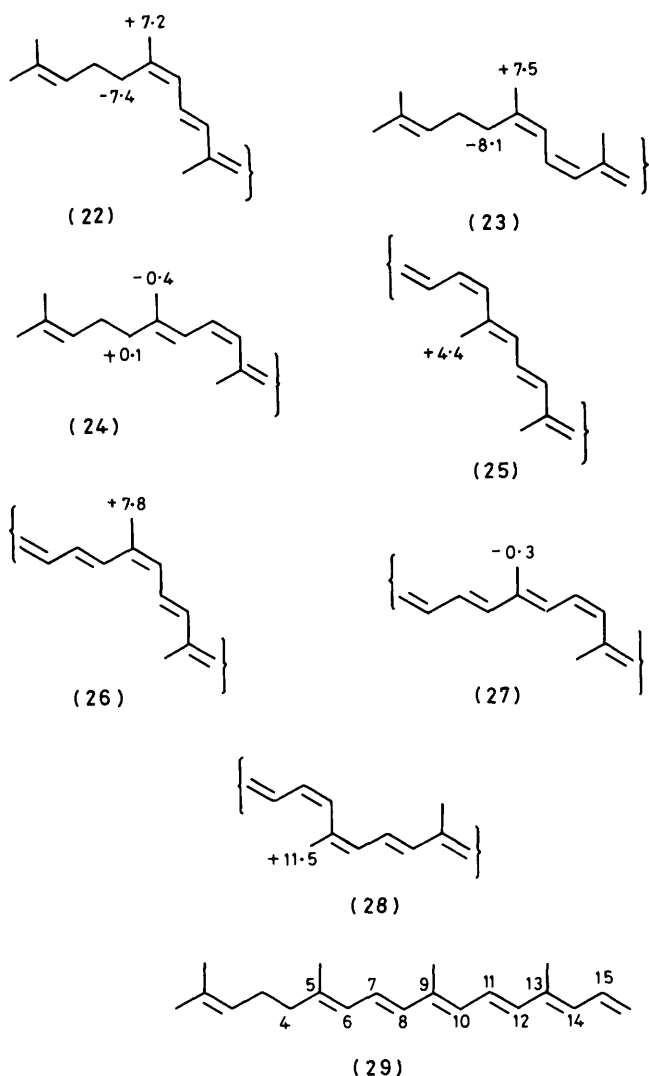rightarrow Z$ stereochemical inversion at the C-11, C-12 double bond gives only a small decrease (shielding) in the chemical shift of the 9-Me, and stereochemical inversions at C-5, C-6 or C-13, C-14 produce shifts (deshielding) at the 9-Me which are too small (ca. 0.1 p.p.m.) to provide reliable data.

^{1}H N.m.r. Spectroscopy.—Without doubt, in those instances where sufficient quantities of material are available, ^{13}C n.m.r. spectroscopy is the most powerful spectroscopic method for assigning the stereochemistry of poly-Z-polyene isoprenoids. However, as the extensive studies of Englert et al.[4d] have demonstrated, when the quantity of material available is at a premium, highfield ^{1}H n.m.r. (400 MHz) spectroscopy can provide valuable information on the structures of Z-retinoids and Z-carotenoids. During our structural studies amongst naturally occurring poly-Z-carotenoid pigments in Tangerine tomatoes,[5] we took the opportunity to examine the use of ^{1}H n.m.r. isomerisation shifts for vinyl methyl group signals in the 100 HMz spectra of geometrical isomers of the model

polyenes (1), (3), and (4). At this frequency the olefinic and the vinyl methylene signals in the various isomers were too complex to be interpreted and used with any accuracy.

As so many research workers have demonstrated before,[11] chemical-shift differences between vinyl methyl resonances associated with isolated Z- (' in-chain '; δ 1.66) and E-double bonds (' out-of-chain '; δ 1.58) provide a valuable internal reference with which to compare other vinyl methyl resonances in the ^{1}H n.m.r. spectra of polyene isoprenoids [see data on formulae (30) and (31) for squalene and 2E,6Z-farnesol respectively].

By contrast, the chemical-shift data obtained for the vinyl methyl groups associated with the terminal double bonds of the chromophore of isomers of the polyenes (1), (3), and (4) [see formulae (32)—(39) for selected data] show that although the shifts are insensitive to the geometry of the double bond (i.e. C-5, C-6) to which they are attached, they are marginally sensitive ($\Delta = -0.03$ p.p.m.) to changes in geometry about the neighbouring (C-7, C-8) double bond. ^{1}H N.m.r. shift data taken from isomers of (1), (3), (4), and other model polyene systems,[12] also show that the vinyl methyl groups positioned deep within the polyene chromophore (i.e. at C-9 and C-13) are sensitive to changes in stereochemistry about neighbouring double bonds (see Figure 2). It is doubtful though whether these data will be of meaningful use for structural purposes since the average isomerisation shifts (see Figure 2) are critically close to the limits of instrument accuracy.

Experimental

For general experimental details, and for details of the preparation of geometrical isomers of the hexaenal (3) and the nonaene (4), see ref. 6.

Carbon-13 magnetic resonance spectra were recorded on a Jeol JNM-PS-100 spectrometer operating at 25.15 MHz, interfaced with a Nicolet 1080 20K computer. Samples were dissolved in deuteriochloroform, which also provided the deuterium lock, and tetramethylsilane was used as an internal reference. The F.I.D.s were compiled using 8 K data points over a spectral width of 6 024 Hz.

Proton magnetic resonance spectra were recorded on a Jeol MH 100 spectrometer; samples were dissolved in deuteriochloroform with tetramethylsilane as an internal reference.

Isomers of Methyl 3,7,11-Trimethyldodeca-2,4,6,10-tetraenoate (1).—(a) A 2 : 1 mixture of the 2Z, 4E, 6E-, and 2Z, 4Z, 6E-isomers of 3,7,11-trimethyldodeca-2,4,6,10-tetraenoic acid was obtained by Wittig condensation between geranyltriphenylphosphonium bromide and 4-hydroxy-3-methylbut-2-enolide. The mixture of acids was esterified with ethereal diazomethane, and the resulting methyl esters were separated by p.l.c. on silica gel using benzene–cyclohexane (9 : 4) as eluant. This gave the following: (i) The 2Z, 4E, 6E-tetraenoate (9) (24%) as an almost colourless oil, R_F (CHCl$_3$) 0.65, $\lambda_{max.}$ (n-hexane) 313 (26 400) and 319infl. nm (25 000); $\nu_{max.}$ (film) 1 705, 1 610, 975, 925, 860, and 845 cm^{-1}; δ$_H$ 7.68 (d, J 15, 4-H), 6.77 (dd, J 15 and 11, 5-H), 6.01 (d, J 11, 6-H), 5.56 (2-H), 5.06 (br, 10-H), 3.62 (OMe), 2.13 and 2.10 (4 H, CH$_2$CH$_2$), 2.0 (3-Me), 1.81 (7-Me), 1.67 (11E-Me), 1.6 (11Z-Me); δ$_C$ see Table; m/z 248 (13%, M), 179 (25%, M − C$_5$H$_9$), and 147 (100%) (Found: M^+, 248.1793. Calc. for C$_{16}$H$_{24}$O$_2$: M, 248.1776). (ii) The 2Z, 4Z, 6E-tetraenoate (10) (14%) as an almost colourless oil, R_F (CHCl$_3$) 0.5, $\lambda_{max.}$ (n-hexane) 312 nm (23 700); $\nu_{max.}$ (film) 1 705, 1 615, 925, 860, and 820 cm^{-1}; δ$_H$ 6.81 (d, J 11, 4-H), 6.39 (apparent t, J 11, 5-H), 6.15 (d, J 11, 6-H), 5.63 (2-H), 5.05 (10-H), 3.63 (OMe) 2.12 (3-Me
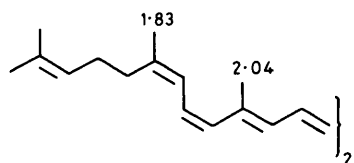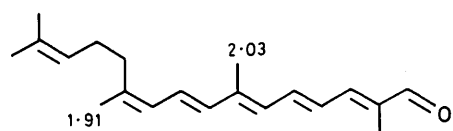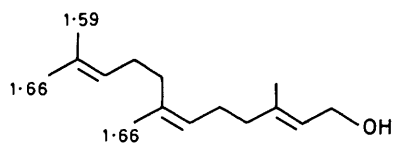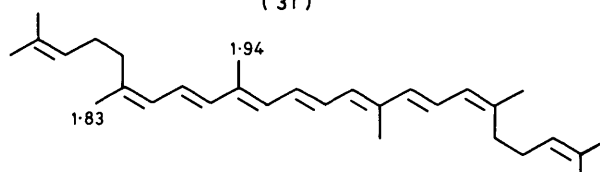
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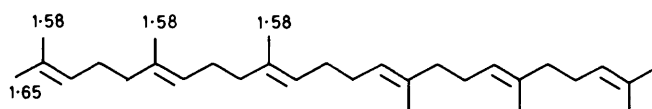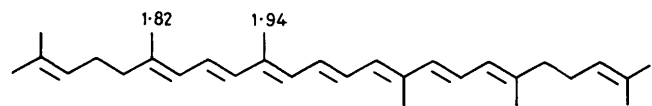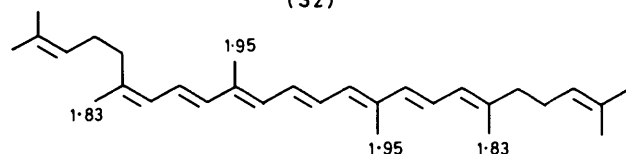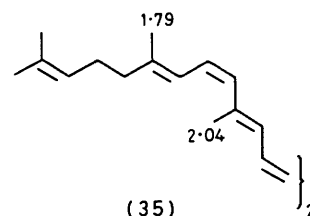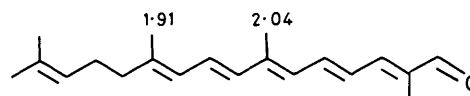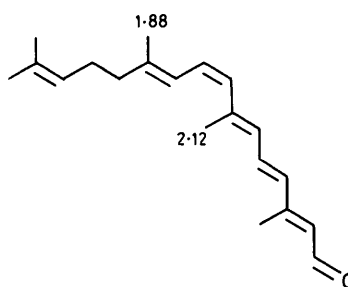
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and CH_2CH_2), 1.79 (7-Me), 1.67 (11E-Me), and 1.6 (11Z-Me); δ_C see Table.

(*b*) A mixture of the 2Z, 4E, 6Z- and all-Z-isomers of the tetraenoate was obtained by Wittig condensation between neryltriphenylphosphonium bromide and 4-hydroxy-3-methylbut-2-enolide, followed by esterification with ethereal diazomethane. Chromatography as under (*a*) gave the following: (i) The 2Z, 4E, 6Z-tetraenoate (11) (19%) as an almost colourless oil, R_F (CHCl$_3$) 0.7, $\lambda_{max.}$ (ethanol) 315 nm; δ_H 7.61 (d, J 15, 4-H), 6.78 (dd, J 15 and 11, 5-H), 6.02 (d, J 11, 6-H), 5.57 (2-H), 5.08 (br s, 10-H), 3.65 (OMe), 2.2 (4 H, CH_2CH_2), 2.01 (3-Me), 1.83 (7-Me), 1.67 (11E-Me), and 1.6 (11Z-Me); δ_C see Table. (ii) The all-Z-tetraenoate (12) (14%) as an almost colourless oil, R_F (CHCl$_3$) 0.5, $\lambda_{max.}$ (ethanol) 309 nm; δ_H 6.83 (d, J 11, 4-H), 6.43 (apparent t, J 11, 5-H), 6.15 (d, J 11, 6-H), 5.66 (2-H), 5.1(br s, 10-H), 3.67 (OMe): 2.18 and 2.14 (4 H, CH_2CH_2), 2.14 (3-Me), 1.83 (7-Me), 1.67 (11E-Me), and 1.6 (11Z-Me); δ_C see Table.
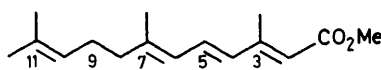
(*c*) A 3 : 2 mixture of 2E, 4E, 6Z- (7) and 2E, 4Z, 6Z- (8) isomers of the tetraenoate was obtained (74%) by Wittig condensation between neryltriphenylphosphonium bromide and E-methyl 3-formylbut-2-enoate. Analytical t.l.c. with numerous solvent systems on silica gel, alumina, and silica gel impregnated with 20% silver nitrate, failed to resolve cleanly the stereoisomers. P.l.c. [developing with benzene–cyclohexane

(9 : 4)] produced a single band, R_F 0.3—0.5, the less strongly adsorbed half of which showed a small (*ca.* 20% by ^{1}H n.m.r. spectroscopy) enrichment with respect to the 2E, 4Z, 6Z-tetraenoate.

By contrast, Horner condensation between neryldiphenylphosphine oxide and E-methyl 3-formylbut-2-enoate gave the 2E, 4E, 6Z-tetraenoate (7) (34%) contaminated only by 5% of the all-E-isomer (by ^{13}C n.m.r. spectroscopy), $\lambda_{max.}$ (n-hexane) 297infl. (28 600), 308 (36 500), and 318infl. (30 900) nm; $\nu_{max.}$ (film) 1 705, 1 605, 960, 925, 880, 830, and 735 cm^{-1}; δ_H 6.78 (dd, J 15 and 11, 5-H), 6.07 (d, J 15, 4-H), 5.89 (d, J 11, 6-H), 5.69 (2-H), 5.06 (br s, 10-H), 3.65 (OMe), 2.31 (3-Me), 2.19, 2.13, 2.11 (m, CH_2CH_2), 1.84 (7-Me), 1.67 (11E-Me), and 1.60 (11Z-Me); δ_C see Table; m/z 248 (16%, M), 182 (73%), 151 (93%), 150 (84%), and 119 (100%) (Found: M^+, 248.1790).

The ^{13}C n.m.r. data collected in the Table for the 2E, 4Z, 6Z-tetraenoate (8) were obtained by comparison of the spectra of pure 2E, 4E, 6Z- and mixed 2E, 4E, 6Z- and 2E, 4Z, 6Z-isomers.

(*d*) A mixture of the all-E- and 2E, 4Z, 6E-isomers of the tetraenoate was obtained by Wittig condensation between geranyltriphenylphosphonium bromide and E-methyl 3-formylbut-2-enoate. Chromatography as under (*a*) gave the following: (i) The 2E, 4Z, 6E-tetraenoate (6) (27%) as an almost colourless oil, $\lambda_{max.}$ (EtOH) 310 nm; $\nu_{max.}$ (film) 1 705,

Table. ^{13}C N.m.r. data (p.p.m. from SiMe$_4$) of the isomeric tetraenoates (5)—(12)

Carbon atom	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
1	167.4	167.4	167.5	167.5	166.7	166.4	166.8	166.5
2	125.0	123.7	125.9	125.0	127.6	123.9	127.3	123.8
3	153.2	153.9	153.3	153.9	151.6	153.0	151.7	153.0
4	133.5	129.7	133.2	130.0	125.7	125.0	125.8	124.8
5	131.1	129.2	131.1	129.0	132.3	129.5	132.3	129.2
6	123.7	121.5	123.7	122.2	123.7	122.0	123.7	122.7
7	143.7	143.7	144.0	144.0	143.9	143.5	144.3	143.9
8	40.4	40.4	33.0	32.3	40.3	40.5	32.9	32.3
9	26.6	26.5	26.9	26.9	26.6	26.5	27.0	27.0
10	117.7	118.5	117.6	118.4	115.7	117.8	115.7	117.8
11	131.8	131.9	132.2	132.2	131.8	131.8	132.3	132.1
11E-Me	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.6
3-Me	13.8	15.9	13.9	19.2	20.9	25.5	21.0	24.7
7-Me	17.1	16.7	24.3	24.4	17.2	16.6	24.3	24.7
11Z-Me	17.7	17.7	17.7	17.7	17.7	17.7	17.7	17.7

[δ 50.7—50.9 p.p.m. (OCH$_3$)].
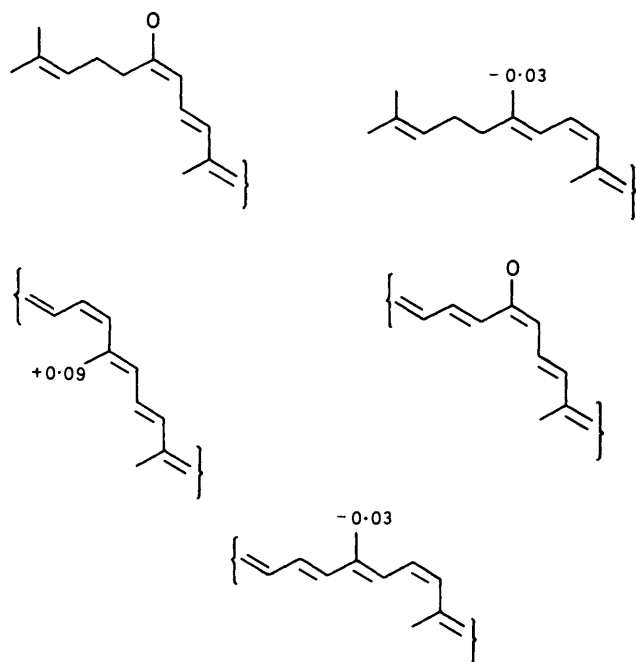
Figure 2. Average values of the ^{1}H n.m.r. isomerisation shifts $\Delta = \delta_Z - \delta_E$ in p.p.m. (CDCl$_3$) for vinyl methyl groups on Z-polyene polyisoprenoids

1 605, and 1 160 cm^{-1}; δ_H 6.5—5.9 (m, 3 H), 5.82 (2-H), 5.08 (br s, 10-H), 3.68 (OMe), 2.30 (3-Me), 2.2—2.1 (m, 4 H), 1.79 (7-Me), 1.68 (11E-Me), and 1.6 (11Z-Me); δ_C see Table. (ii) The all-E-tetraenoate (5) (55%) which showed identical u.v., i.r., and ^{1}H n.m.r. spectral data to those published previously; δ_C see Table.

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