Pure & Appl. Chem., Vol. 47, pp. 97–102. Pergamon Press, 1976. Printed in Great Britain.

CARBON-13 NMR SPECTRA OF CAROTENOIDS

G. P. Moss
Department of Chemistry, Queen Mary College, Mile End Road, London, E1 4NS, UK

Abstract—The ^{13}C-NMR spectrum of β-carotene has been assigned using data from deuterium labelled samples, and *cis* vitamin A. A study of methyl β-apo carotenoates is consistant with this result. Spectra of derivatives of β-carotene, such as zeaxanthin, isozeaxanthin, violaxanthin and alloxanthin, have been analysed.

The stereochemistry of lutein is deduced from its spectrum to be *trans* between the 3'-hydroxyl group and the polyene chain at C-6'. It is suggested that isomytiloxanthin has the alkyl groups at C-5 and C-6 of the cyclohexanone ring *trans*.

Cis double bonds give characteristic changes in the spectrum compared with that of the corresponding *trans* compound violeoxanthin is shown to be the 9-*cis* isomer of violaxanthin. ^{13}C–^{31}P coupling over six bonds is observed with *trans* β-C_{15} Wittig salt, but only over four bonds with the *cis* isomer. The 15,15'-*cis*-β-carotene spectrum suggests a revision of the assignment of the phytoene spectrum.

Using the data from appropriate apo esters and other model compounds the spectra of capsorubin and azafrin are analysed. The absence of suitable model compounds as yet prevents a complete assignment of the fucoxanthin spectrum.

INTRODUCTION

One of the most important techniques for the characterisation, or structure determination of carotenoids is ^{1}H-NMR spectroscopy. A recent review[1] listed the readily identifiable signals from eighty different end groups occurring in natural carotenoids. Due to complex second order spin–spin coupling in most cases only methyl signals are clearly distinguished without the use of the higher resolution possible with a 220 MHz spectrometer or similar instrument. Thus characterisation often relies on only two or three signals per end group.

In principal ^{13}C-NMR spectroscopy is a much more powerful technique for structure determination.[2] Even though the first spectrum was observed in 1957 by Lauterbur the low natural abundance of ^{13}C (1.1%) and low sensitivity compared with ^{1}H (1.59%) has delayed its routine application until recently. The use of large sample tubes, CATed, pulsed spectra with Fourier transformation, and broad band proton decoupling allows ready measurement of ^{13}C-NMR spectra. As yet, either quite large amounts (e.g. 100 mg) of pure carotenoids or long accumulation times (e.g. 24 hr) are needed. Once obtained, the spectra are relatively easy to interpret, although assignment may be difficult. Each signal is normally a sharp singlet with ^{1}H–^{13}C spin–spin coupling absent due to the decoupling, and ^{13}C–^{13}C spin–spin coupling absent because of the low probability of two ^{13}C-atoms being adjacent to each other.

A number of different techniques may be used to assign ^{13}C-NMR spectra. Characteristic chemical shift values may often be used to identify some signals (see Fig. 1) and will readily permit the separation of the signals into two groups, those derived from sp^2 and those from sp^3 hybridised carbon atoms. Data derived from model compounds is obviously useful. The number of hydrogen atoms attached to each carbon atom may be determined by re-running the spectrum under single frequency off-resonance (SFOR) conditions whereby a small residual coupling is observed between each proton and the carbon it is attached to. Thus methyl carbon signals appear as quartets, methylenes as triplets etc. Under carefully controlled conditions additional information may be obtained from SFOR conditions by means of second-order effects. Thus with α-ionone not only could C-7 be related to C-8 using their ^{1}H–^{1}H spin–spin coupling, but also it was possible to relate C-7 to C-6.[3] Probably the most unambiguous method of assignment is by selective deuteration. A carbon atom so labelled will not normally be detected in the ^{13}C-NMR spectrum. It was by this means that C-15 of β-carotene was first assigned correctly.[4]

β-CAROTENE

The ^{13}C-NMR spectrum of β-carotene (I) and some related compounds was reported by Roberts and co-workers in 1970.[5] Extensive studies since then have confirmed their assignment of the sp^3 signals, but, as deuteration[4] and SFOR studies[6] showed, the sp^2 signals need revision. It has become apparent that the assignment of polyene sp^2 signals cannot be based on simple models.

The spectrum of vitamin A acetate can be readily related to β-carotene (Fig. 2). The only significant differences occurring with C-10, C-12, C-13, C-14 and, of course, C-15. We were able to assign C-11 and C-12 (but not between them) by means of [11,12-$^{2}H_2$] vitamin A acetate and the associated isotope effect was noticed at C-9, C-10, C-13 and C-14. SFOR data identified C-5, C-6, C-9, C-13 which can also be recognised in the high resolution spectrum (Fig. 2) by their narrower line widths. The various alternative assignments were distinguished by the study of a number of *cis* isomers at the trisubstituted double bond. Methyl bixin and its all *trans* isomer (XX) was particularly diagnostic due to the strong polarisation of the polyene

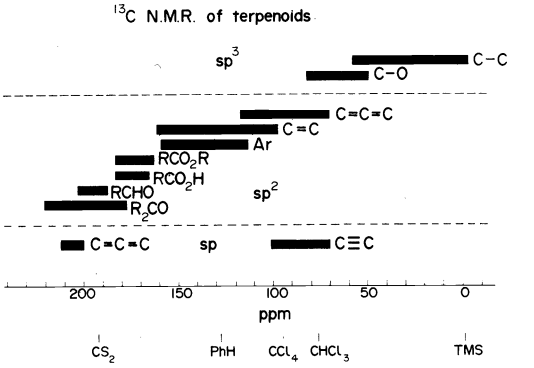


Fig. 1. Chemical shifts observed in terpenoids (ppm from $\delta_{TMS} = 0$) according to hybridisation of the carbon atom (top sp^3, middle sp^2, bottom sp).

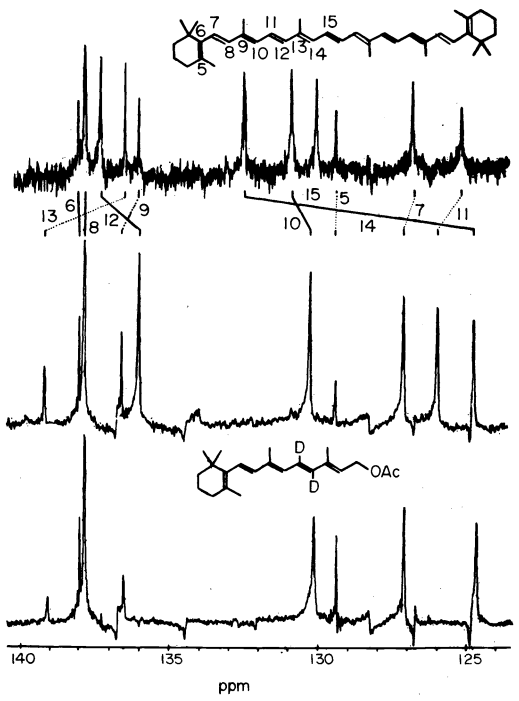


Fig. 2. sp^2 Region of the ^{13}C-NMR spectra of β-carotene (top curve), vitamin A acetate (middle curve) and [11,12-$^2H_2$] vitamin A acetate (bottom curve).

chain by the carbonyl group (Table 1). Using these data the assignment of 9-*cis* and 13-*cis* vitamin A acetate is quite straightforward, and hence also β-carotene (Table 1). These results also demonstrate the relatively constant changes observed between the spectra of *cis* and *trans* isomers.

β-APO CAROTENOIDS

The assignment of the β-carotene spectrum was confirmed by a study of a series of methyl β-apo carotenoates (Fig. 3). Polarisation of the polyene chain by the carbonyl group results in strong shielding of the α-position (and progressively less at γ, ϵ etc.) and strong deshielding at the β-position (and progressively less at δ, ξ etc.). β-Carotene thus represents the limiting values for these signals. In most cases SFOR data was used to confirm C-5, C-6, C-9 and C-13. Some variation in the signals was observed, possibly due to concentration effects.[3]

A limited study by Bremser and Paust[7] of a number of β-apocarotenals and ketones came to similar conclusions (except for C-9 and C-13). Detailed studies of the ^{13}C-NMR spectrum of retinal by Rowan and Sykes,[8] and by Becker *et al.*[9] also show minor variations from Bremser and Paust[7] in the assignment of C-10, although there are solvent differences (See also ref. (27)). The differences in chemical shift between spectra run in acetone and cyclohexane[8] parallel our experience of concentration and solvent effects[3] where the significant changes occur at the carbonyl and β-signal, and less so with the δ- and ξ-signals. Although the α-signal may be just affected most other signals are unchanged. The original assignment of α- and β-ionone[5] has been revised by Hollenstein and von Philipsborn[10] and later workers.[8,11]

ZEAXANTHIN, ISOZEAXANTHIN, ISOCRYPTAXANTHIN, VIOLAXANTHIN AND ALLOXANTHIN

The effect on the ^{13}C-NMR spectrum of substitution of a cyclohexane ring by a hydroxyl group has been studied by Roberts *et al.*[12] Using his parameters the spectrum of zeaxanthin (II) is readily interpreted (Table 1). As expected the changes from the spectrum of β-carotene imply a predominantly equatorial hydroxyl group. Similarly the spectrum of isozeaxanthin (III) also can be readily assigned (Table 1). In this case a pseudo axial hydroxyl group is suggested, possibly implying that when the hydroxyl

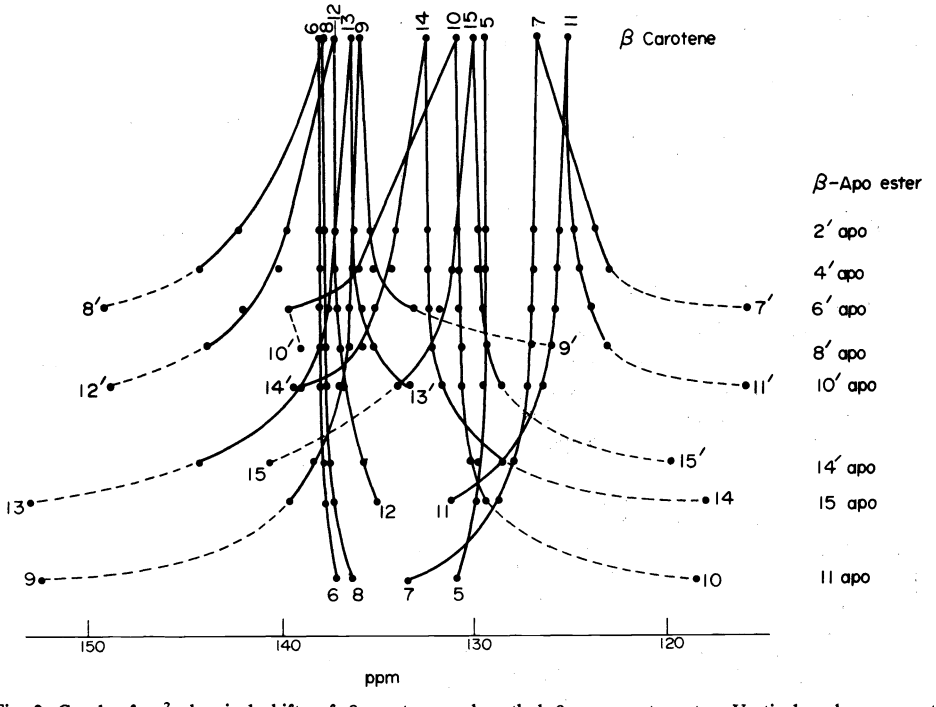


Fig. 3. Graph of sp^2 chemical shifts of β-carotene and methyl β-apo carotenoates. Vertical scale represents increasing number of double bonds (n) with β-carotene effectively $n = \infty$.

Table 1. ^{13}C-NMR chemical shifts of carotenoids ($\delta_{TMS} = 0$)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	1-Me's		5Me	9-Me	13-Me	Other signals
β-Carotene (I)‖	34.3	39.7	19.3	33.2	129.3	138.0	126.7	137.8	136.0	130.8	125.0	137.3	136.4	132.4	130.0	29.0		21.7	12.7	12.7	
15,15′-*cis*-β-carotene‖	34.2	39.7	19.3	33.1	129.4	138.0	126.8	137.8	136.3	130.8	125.5	137.4	137.1	126.8	125.5	29.0		21.8	12.7	12.5	
15,15′-dehydro-β-carotene (ref. 5)§	34.7	40.3	20.2	33.7	129.6	138.3	127.3	138.3	135.5	131.1	127.3	137.2	146.4	111.6	99.3	29.3		22.1	13.0	15.5	
Iso cryptoxanthin (IV) β end	34.6	39.7	19.3	33.2	129.5	137.9	126.8	137.9	136.2	131.0	125.3	137.3	136.4	132.5	130.0	29.0		21.8	12.8	12.8	
Iso cryptoxanthin (IV) OH end	34.6	35.0	28.6	70.5	129.8	142.1	125.7	139.0	135.5	131.8	124.8	137.9	136.7	132.9	130.3	27.6	29.0	18.8	12.8	12.8	
Isozeaxanthin (III)	34.6	34.6	28.5	70.2	129.8	142.0	125.6	138.9	135.5	131.7	124.8	137.8	136.5	132.7	130.1	27.5	29.1	18.7	12.7	12.7	
Zeaxanthin (II)‡‖¶	37.1	48.2	65.1	42.4	126.1	137.6	125.5	138.5	135.7	131.3	124.9	137.6	136.5	132.6	130.0	28.7	30.2	21.7	12.8	12.8	
Lutein (VII)‡ β-end	37.1	48.4	65.1	42.5	126.2	137.6	125.6	138.5	135.6	131.3	124.9	137.6	136.5	132.6	130.0	28.7	30.2	21.6	12.7	12.7	
Lutein (VII)‡ α-end	34.0	44.7	65.9	125.6	137.8	55.0	128.6	137.8	135.0	130.8	124.5	137.6	136.5	132.6	130.0	24.3[a]	29.5[a]	22.8[a]	13.2	12.7	
Violaxanthin (V)	35.4	47.2	64.1	40.9	67.3[a]	70.4[a]	123.9	137.2	134.2	132.4	124.6	138.2	136.4	132.9	130.2	24.7	29.7	20.0	12.8	13.1	
Violeoxanthin (9-*cis* V)‡ *trans* end	35.4	47.3	64.2	41.0	67.2[a]	70.3[a]	123.8	137.3	134.2	132.3	124.6	138.2	136.3	132.8	130.7[b]	24.9	28.7	20.0	12.8	13.1	
Violeoxanthin (9-*cis* V)‡ *cis* end	35.4	47.3	64.2	41.0	67.8[a]	70.6	126.0	129.2	132.6	130.8	123.6	137.3	136.7	132.6	130.0[b]	24.9	29.7	20.0	21.1	13.1	
Alloxanthin (VI)	36.9	46.7	64.8	41.5	137.2[e]	124.3[e]	89.1[a]	98.8[a]	119.1	138.0[e]	124.3[e]	135.2[e]	136.4[e]	133.4[e]	130.3[e]	28.8	30.5	22.4	18.0	12.7	
Isomytiloxanthin (XI)‡ acetylene end	37.0	46.9	65.0	41.7	138.2	124.6	n.d.	n.d.	†	†	†	†	†	†	†	28.9	30.8	22.8	18.2	13.0	
Isomytiloxanthin (XI)‡ keto end	41.8	50.8	208.6	48.3	40.7	84.1	n.a.	201.0	†	†	†	†	†	†	†	29.4	31.2	23.7	12.1	14.9	
Capsorubin (XIX)¶	44.0	50.8	70.3	45.3	59.0	203.0	121.1	146.8	134.0	140.6	124.7	141.8	136.9	134.9	131.2	25.1[a]	25.9[a]	21.3[a]	12.8	12.8	
Canthaxanthin (XVIII)‖	35.7	37.7[f]	34.3[f]	198.7	129.9	160.9	124.2	141.1	134.8	134.3	124.7	139.3	136.6	133.6	130.5	27.7		13.7	12.5	12.7	Acetate
Fucoxanthin (XXII) keto end	35.2[a]	47.3[b]	64.2	40.9[b]	67.2[c]	72.6[c]	41.8[b]	197.9	134.5	139.2	123.4	145.1	135.4	136.7	129.5	25.0[d]	29.2[d]	21.5[d]	11.9[e]	14.0[e]	
Fucoxanthin (XXII) allenic end	35.8[a]	45.4[b]	68.2	45.5[b]	66.2[c]	117.6	202.4	103.4	132.6	128.5	125.7	137.1	138.1	132.2	132.6	31.3[d]	32.2[d]	28.2[d]	13.0[e]	12.7[e]	Me 21.2, CO 170.5
All *trans* phytoene (ref. 21)	131.4	124.2[a]	26.9	39.9	135.1[b]	124.5[a]	26.9	39.9	135.5[b]	124.7[a]	26.9	40.7	138.3	125.7	127.3	17.8	25.8	16.2	16.2	16.9	
cis Phytoene (ref. 21)	131.4	124.2[a]	27.0	39.9	135.1[b]	124.5[a]	27.0	39.9	135.5[b]	124.7[a]	27.0	40.7	139.6	120.5	123.6	17.8	25.8	16.2	16.2	16.7	
Methyl azafrin (XXI) diol end	38.7	36.4	18.0	36.4	75.4	79.6	130.6	134.7	135.8	131.4	126.2	137.4	138.9	131.9	133.9	26.6	27.1	25.2	13.3	13.0	
Methyl azafrin (XXI) apo end										168.0	116.0	149.0	133.7	139.4	128.8					12.8	MeO 51.5
All *trans* methyl bixin (XX)						167.9	115.9	149.0	133.5	139.4	124.4	141.7	136.8	134.8	131.1				12.6	12.6	MeO 51.5
Methyl bixin (9-*cis* XX) *trans* end						167.9	115.9	149.1	133.5	139.5	124.3	141.9	136.6[a]	135.0	131.3				12.6	12.8	MeO 51.5
Methyl bixin (9-*cis* XX) *cis* end						168.1	117.6	140.5	131.6	138.0	123.3	140.5	137.0[a]	134.3	130.8				20.3	13.1	MeO 51.7
Crocetindial								194.2	137.4[a]	148.4	124.0	145.4	138.0[a]	136.7	132.2				9.7	12.8	

[a,b,c,d]Assignments may be reversed.

[e]Assignments may need revision due to partial interference by signal from C_6F_6 used as internal ^{19}F lack.

[f]Assignment confirmed by Dr. J. Lauterwein (Institute du Radium, Orsay, France) using INDOR.

n.a. = Not assigned.

n.d. = Not detected.

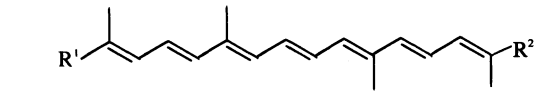
†Signals obscured by signal from C_6F_6 used as internal ^{19}F lack.

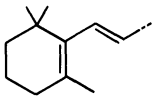
‡No SFOR data, assignment based on data from model compounds.

§Data calculated from ref. 5 by $\delta^{TMS} = 192.8\text{-}\delta^{CS_2}$.

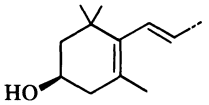
‖See also G. Englert, *Helv. Chim. Acta*, **58**, 2367 (1975), where data will be found for ε-carotene, astaxanthin, astacene, crustaxanthin tetraacetate and other derivatives.

¶See also J. Szabolcs, *Pure Appl. Chem.* **47**, 147–159 (1976).

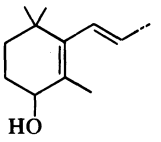




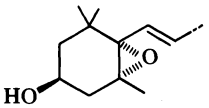
a



b

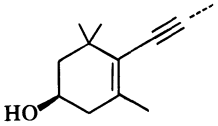


c

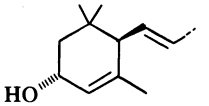


d

I: $R^1 = R^2 = a$
II: $R^1 = R^2 = b$
III: $R^1 = R^2 = c$
IV: $R^1 = a$, $R^2 = c$
V: $R^1 = R^2 = d$
VI: $R^1 = R^2 = e$
VII: $R^1 = b$, $R^2 = f$



e



f

group is in a pseudoequatorial conformation it eclipses the 5-methyl group. With the symmetric carotenoids considered above there are only half as many carbon signals as would be expected with a pigment such as isocryptoxanthin (IV). However the spectrum is exactly as expected from a combination of the spectra found for each end group. Violaxanthin (V), the natural diepoxide of zeaxanthin, only shows small spectral changes except for C-5 and C-6 (Table 1). Similarly the 7,8-dehydro system of alloxanthin (VI) only slightly modifies the position of the sp^3 signals of zeaxanthin, and shows typical changes in the sp^2 region (cf. 15,15'-dehydro-β-carotene, Table 1).

LUTEIN AND ISOMYTILOXANTHIN

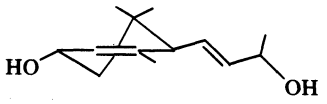
The results discussed above are only concerned with the assignment of signals from the spectra of fully characterised carotenoids. However, ^{13}C-NMR spectroscopy can provide stereochemical and structural information. As I reported at Cluj[13] the problem of the stereochemistry and conformation of the 3' and 6' substituents of lutein (VII) *trans* model (VIII) show that the alkyl (polyene) group at C-6 is equatorial, and with the *cis* model IX axial (Table 2). The effect at C-2 is particularly diagnostic. These conclusions have since been confirmed by several groups.[15–17]

A tentative assignment of isomytiloxanthin (XI) was reported at Cluj[18] (Table 1). It was based on data from the model compounds XII and XIII with the stereochemistry shown. This data, ^{1}H-NMR studies and the biogenesis suggest that isomytiloxanthin has the opposite stereochemistry at C-6 from the model compounds.

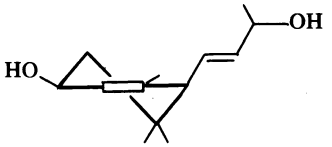
CIS ISOMERS OF CAROTENOIDS AND RELATED COMPOUNDS

Cis isomers were used in the full assignment of β-carotene (*vide supra*). The characteristic shifts associated with a *cis* trisubstituted double bond are summarised on formula XV.

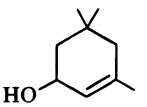
Violeoxanthin was shown by Szabolcs and Tóth[19] to be a *cis* isomer of violaxanthin (V). The position of the *cis* double bond was most clearly demonstrated to be at C-9, C-10 by the ^{13}C-NMR spectrum of violeoxanthin[20] (Table



VIII



IX



X

can be demonstrated from a comparison of the spectrum with model compounds. Both isomers of 3-hydroxy-α-ionol† (VIII and IX) were prepared and one related to the ε-end of lutein by both ^{1}H and ^{13}C NMR spectroscopy (Table 2). The β-end of lutein gave signals which were readily identified using the spectrum of zeaxanthin (Table 1). Lutein and the model compounds must have a preferred conformation with the 3-hydroxy groups equatorial to minimise interactions with the 1-methyls. Comparison of the spectrum of α-isophorol (X) with those of lutein and the 1); especially by the characteristic shifts of the 9-methyl and C-8 signals.

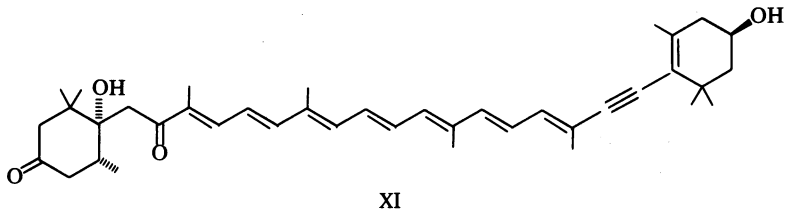
In a study of the β-C_{15}-Wittig salt (XIV) the spectrum is complicated by the additional phenyl signals, and the presence of ^{13}C–^{31}P spin–spin coupling. In order to pair the signals split by ^{13}P the spectra were run at both 22.63 MHz and 25.15 MHz so that changes in the positions of the signals could be distinguished from the constant coupling constants. Both the 9-*cis* and 9-*trans* Wittig salt were examined and typical changes observed. However the most interesting feature of these spectra were the long range ^{13}C–^{31}P couplings. With the zig-zag π system of the *trans* isomer the coupling is transmitted over up to six

†Carotenoid numbering.

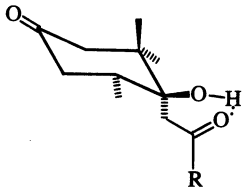
Table 2. ^{13}C-NMR data for the ε-end of lutein and related models

		1	2	3	4	5	6
δ	Lutein (VII)	34.0	44.7	65.9	125.6	137.8	55.0
δ	*trans* diol (VIII)	34.2	45.5	66.4	125.9	137.8	55.0
δ	*cis* diol (IX)	35.2	41.3	67.3	126.0	138.0	55.0†
δ	α-isophorol (X)	31.3	45.4‡	66.7	124.7	135.2	44.7‡
Δδ	VII–X	2.7	−0.7	−0.8	0.9	2.6	10.3
Δδ	VIII–X	2.9	0.1	−0.3	1.2	2.6	10.3
Δδ	IX–X	3.9	−4.2	0.6	1.3	2.8	10.3
Δδ	6-equatorial Me (ref. 14)	9.03	0.05	−0.22	0.05	9.03	5.96
Δδ	6-axial Me (ref. 14)	5.41	−6.37	−0.06	−6.37	5.41	1.40

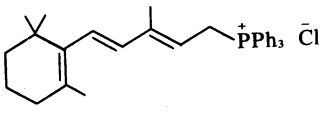
†Average of two signals (54.9, 55.1), due to diastereoisomers differing at C-9.
‡Assignment may be reversed.



XI



XII: R = H
XIII: R = Et



XIV

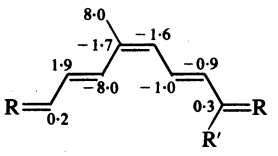
bands. In contrast the *cis* double bond of the other isomer does not allow such an extended zig–zag chain so that coupling only occurs over four bonds (Table 3).

The other relatively stable positions for a *cis* double bond is at 15,15′. To examine the effect of isomerisation at this position the spectra of 15,15′-*cis*-β-carotene and a [15,15′-$^2H_2$] labelled sample were analysed. C-15 was readily identified by the 'missing' signals in the spectrum of the labelled material, and comparison with the data for all *trans*-β-carotene readily suggested a complete assignment (Table 1). The changes are summarised on formula XVI. These figures show that the assignments by Granger *et al.*[21] of C-13 and C-15 of the phytoene isomers should be reversed. The remaining ambiguities left with the phytoene spectra are not resolved even with the extensive studies of related systems by Crombie *et al.*[22]

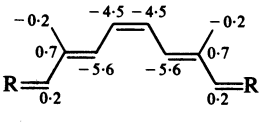
The changes observed[8,9] between *trans* retinal and the hindered 11-*cis* isomer (see formula XVII) show that the position of any isolated *cis* double bond should be readily recognised from the ^{13}C-NMR spectrum.

CANTHAXANTHIN, CAPSORUBIN, AZAFRIN AND FUCOXANTHIN

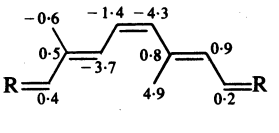
The effect of a carbonyl group on the spectra of methyl β-apo-carotenoates (Fig. 3) has already been commented on. A keto group at C-4 and C-4′ of β-carotene (canthaxanthin XVIII) polarises the polyene chain and greatly assists the assignment of the sp^2 signals (Table 1), while data from model compounds[23,24] supports the sp^3 assignments. The chromophore of capsorubin (XIX) is essentially the same as *trans* methyl bixin (XX). Comparison of their spectra (Table 1) shows this similarity apart from slight differences in the signals of carbon atoms adjacent to the carbonyl group. The remaining sp^3 carbons are assigned by comparison with the spectrum of helicobasidin[25] and related models.



XV



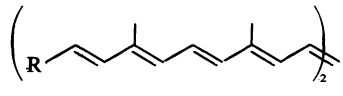
XVI



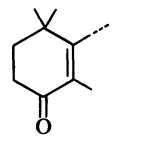
XVII

Table 3. ^{13}C-NMR data for β-C_{15} Wittig salt

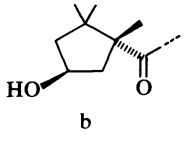
	6	7	8	9	10	11	9-Me
δ 9-*trans* XIV	139.9	128.6	135.4	143.7	112.5	24.7	12.8
δΔ (9 *cis*-9 *trans*)	−2.2	3.2	−7.1	−2.5	0.8	−1.1	8.1
$J_{^{13}C^{31}P}$ (Hz)9-*trans*	2.4	5.0	6.0	14.1	11.3	49.2	2.5
$J_{^{13}C^{31}P}$ (Hz) 9-*cis*			2.5	12.5	11.3	51.3	



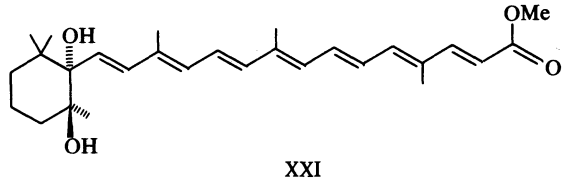
XVIII: R = a
XIX: R = b
XX: R = CO_2Me



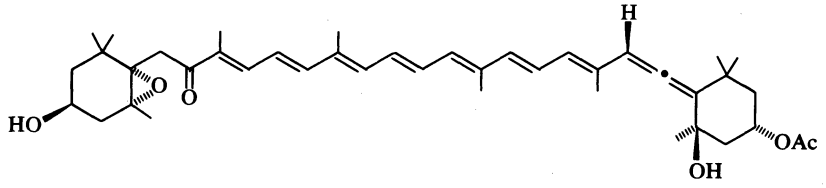
a



b



XXI



XXII

Methyl β-apo-10′-carotenoate is a good model for the assignment of the sp^2 region of the spectrum of methyl azafrin (XXI). In order to identify the sp^3 signals an analysis of the substituent effects expect[12,14,26] for the cyclohexane ring with a diaxial glycol conformation[18] were calculated and found to predict the observed values (Table 1) quite closely.

Fucoxanthin (XXII) is an interesting case where 41 of the 42 expected signals are clearly resolved.[1] The expected effect of the carbonyl group and SFOR data readily allows an assignment of the sp^2 signals. However apart from the division into categories using SFOR data there are too few spectra derived from model compounds to assist the assignment of either end group.

Acknowledgements—Finally I should like to thank those who have been associated with this work; in particular Professor B. C. L. Weedon for his encouragement and the supply of precious samples, Professor E. W. Randall for access to the Bruker HFX 90 spectrometer and my principal collaborator in this work, Mr. J. F. Hagel. My thanks are also due to Dr. P. S. Pregosin for some of the earlier spectra; Mr. G. M. Leeder, Roche Products Ltd., Welwyn Garden City, (25.15 MHz spectra, of the C_{15} Wittig salts and samples); Dr. G. M. Clarke, Roche Products Ltd., Welwyn Garden City, (*cis* vitamin A acetates); Dr. O. Isler, Hoffman-La Roche & Co. Ltd., Basle, ([11,12-$^2H_2$] vitamin A acetate); Dr. A. Khare, (preparation of isomytiloxanthin models); Mr. D. Goodfellow (preparation of lutein models); Miss E. A. H. Hall (preparation of [15,15′-$^2H_2$] 15,15-*cis*-β-carotene).

REFERENCES

[1]G. P. Moss and B. C. L. Weedon, in *Chemistry and Biochemistry of Plant Pigments*. 2nd Edn, Chap. 3 (editor T. W. Goodwin), Academic Press, New York (1976).
[2]G. C. Levy and G. L. Nelson, *Carbon-13 Nuclear Magnetic Resonance for Organic Chemists*. Wiley Interscience, New York (1972); J. B. Stothers, *Carbon-13 NMR Spectroscopy*. Academic Press, New York (1972); L. F. Johnson and W. C. Jankowski, *Carbon-13 NMR Spectra*. Wiley Interscience, New York (1972); E. Breitmaier and W. Voelter, ^{13}C *NMR Spectroscopy*. Verlag Chemie (1974).
[3]J. F. Hagel and G. P. Moss, unpublished results.
[4]W. Vetter, G. Englert, N. Rigassi and U. Schwieter, in *Carotenoids*. Chap. 4, (editor O. Isler), Birkhäuser, Basle (1971).
[5]M. Jautelat, J. B. Grutzner and J. D. Roberts, *Proc. Nat. Acad. Sci. U.S.A.* **65**, 288 (1970).
[6]W. Bremser, H. D. W. Hill and R. Freeman, *Messtechnik*, **79**, 14 (1971).
[7]W. Bremser and J. Paust, *Org. Mag. Res.* **6**, 433 (1974).
[8]R. Rowan III and B. D. Sykes, *J. Am. chem. Soc.* **96**, 7000 (1974).
[9]R. S. Becker, S. Berger, D. K. Dalling, D. M. Grant and R. J. Pugmire, *J. Am. chem. Soc.* **96**, 7008 (1974).
[10]R. Hollenstein and W. von Philipsborn, *Helv. Chim. Acta*, **55**, 2030 (1972).
[11]A. K. Bose and P. R. Srinivasan, *Tetrahedron Lett.* 1571 (1975).
[12]J. D. Roberts, F. J. Weigert, J. I. Kroschwitz and H. J. Reich, *J. Am. chem. soc.* **92**, 1338 (1970).
[13]D. Goodfellow, G. P. Moss and P. S. Pregosin, unpublished results.
[14]D. K. Dalling and D. M. Grant, *J. Am. chem. Soc.* **94**, 5318 (1972).
[15]R. Buckecker, P. Hamm and C. H. Eugster, *Helv. chim. Acta*, **57**, 631 (1974).
[16]A. G. Andrewes, G. Borch and S. Liaaen-Jensen, *Acta chem. Scand.* **B28**, 139 (1974).
[17]B. C. L. Weedon, *Pure Appl. Chem.* **47**, 161–171.
[18]B. C. L. Weedon, *Pure Appl. Chem.* **35**, 113 (1973).
[19]J. Szabolcs and Gy. Tóth, *Acta chim. Acad. Sci. Hung.* **63**, 229 (1970).
[20]G. P. Moss, J. Szabolcs, Gy. Tóth and B. C. L. Weedon, *Acta chim. Acad. Sci. Hung.* **87**, 301 (1975).
[21]P. Granger, B. Maudinas, R. Herber and J. Villoutreix, *J. Mag. Res.* **10**, 43 (1973).
[22]L. Crombie, R. W. King and D. A. Whiting, *J. Chem. Soc. Perkin* I, 913 (1975).
[23]F. J. Weizert and J. D. Roberts, *J. Am. chem. Soc.* **92**, 1347 (1970).
[24]G. Magnusson and S. Thorén, *J. Org. Chem.* **38**, 1380 (1973).
[25]M. Tanabe, K. T. Suzuki and W. C. Janowski, *Tetrahedron Lett.* 4723 (1973).
[26]H. Ziffer, J. I. Seeman, R. J. Highet and E. A. Sokoloski, *J. Org. Chem.* **39**, 3698 (1974).
[27]Y. Inoue, A. Takahashi, Y. Tokitô, R. Chûjô and Y. Miyoshi, *Org. Mag. Res.* **6**, 487 (1974).