Application of the Tris(dipivalomethanato)europium(III) Nuclear Magnetic Resonance Shift Reagent to Carotenoids

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The tris(dipivalomethanato)europium(III) nuclear magnetic resonance shift reagent has been applied to a number of selected carotenoids of known structure. The results are compatible with those obtained for other classes of organic compounds, and the utility of this technique in carotenoid NMR spectrometry has been demonstrated.

The lanthanide shift technique is now well established in organic NMR spectrometry. Since Hinckley [1] discovered that the bispyridine adduct of tris(dipivalomethanato)europium(III) caused dramatic downfield shifts in the NMR spectrum of cholesterol, several investigators have been looking into the mechanistic action of lanthanide shift reagents. [2-13] Other workers have applied this technique to structural problems of diverse character, [14-28] and their success clearly demonstrates the utility of the shift reagents.

It seems established that the shifts are induced by pseudocontact interaction between the metal reagent and functions in the substrate possessing lone pair electrons. The signals observed appear to be an average of the signals from coordinated and uncoordinated substrate due to a rapid (on the NMR scale) equilibrium, [2,6] as indicated by concentration dependency. [2]

A number of metal chelates other than tris(dipivalomethanato)-europium(III), abbreviated Eu(dpm)$_3$, have been tested as shift reagents, [2,4,11,13,23,26,27] but the interest is now centered around europium(III) and praseodymium(III) chelates due to their superior properties regarding large shift values and minimum signal broadening. Praseodymium chelates induce shifts in the opposite (*i.e.* upfield) direction to europium chelates. [11]

The nature of the ligand governs the properties of the chelates to a great extent [6] and until quite recently dipivaloylmethane (dpmH) gave chelates with the best solubility in non-polar solvents. After the present study Rondeau and Sievers [29] have demonstrated that 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione (fodH) gives reagents of superior properties (*i.e.* solubility and acidity) to those obtained with dpmH.

We have applied the shift technique using Eu(dpm)$_3$ to selected carotenoids of known structure and we now report the results obtained for this class of compounds.
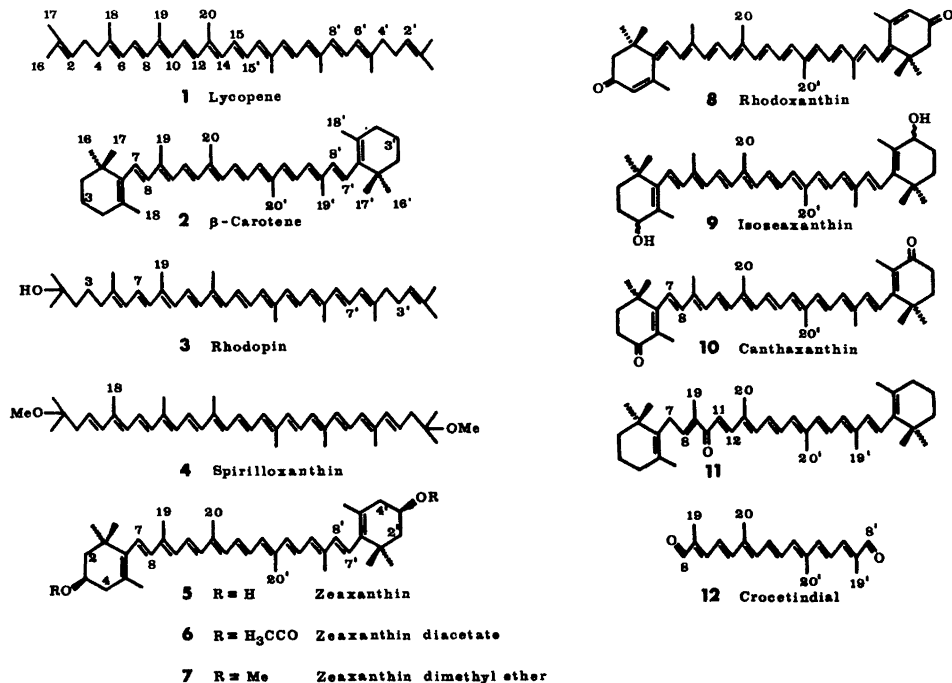
RESULTS AND DISCUSSION

NMR spectrometry of carotenoids was originally systematized by Barber et al.[31] Most carotenoid NMR spectra have been recorded at 60 MHz although some have been recorded at 100 MHz and 220 MHz.[32,33] Spin decoupling has had limited application in carotenoid NMR problems.[34]

Carotenoid NMR spectra in general exhibit signals in two main regions: τ 7.7 − 9.2 (methyl, methylene, and methine signals) and τ 3.0 − 4.6 (olefinic signals). The resolution of these signals usually allows only the methyl signals to be identified. Only a few functional groups give rise to signals outside these regions (prim. and sec. alcohols, aldehydes, acids, esters, methyl ethers, and glycosides).

The lanthanide shift technique appeared very promising for carotenoid NMR problems since diagnostically important signals might be shifted out of their crowded envelopes.

In the following the term "chemical shift" is used in the usual sense, whereas "induced shift" designates the chemical shift of a given proton induced

1 Lycopene

2 β-Carotene

3 Rhodopin

4 Spirilloxanthin

5 R = H   Zeaxanthin

6 R = H$_3$CCO Zeaxanthin diacetate

7 R = Me   Zeaxanthin dimethyl ether

8 Rhodoxanthin

9 Isoseaxanthin

10 Canthaxanthin

11

12 Crocetindial

by the reagent with the unshifted signal as reference. "Relative shift" [8] is the induced shift of a given proton relative to the induced shift of a selected proton.

The carotenoids investigated (structures 1−12) show a fair selection of functionalities.

The hydrocarbons lycopene (1) and β-carotene (2) gave no induced shifts with Eu(dpm)₃, consistent with earlier findings that olefinic and aromatic hydrocarbons do not coordinate.[6,7]

The induced shift dependency on the reagent/substrate ratio has been demonstrated [2,9] but due to solubility problems this has not previously been investigated beyond 2.2 relative moles per complexing function. In some cases more or less constant shift values have been reached at ratios between 1.5 and 2.2.[8,29] Fig. 1 shows a plot of the chemical shifts oberved for the monofunctional
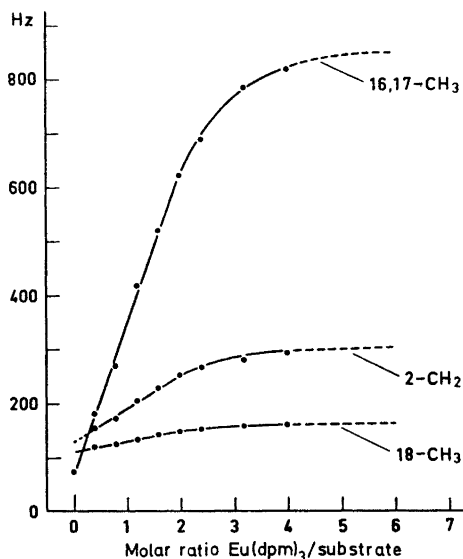

Fig. 1. Chemical shifts (Hz) for the 16,17, and 18-methyl groups and the 2-methylene group of rhodopin (3) with increasing concentrations of Eu(dpm)₃ at constant volume. Reference: TMS.
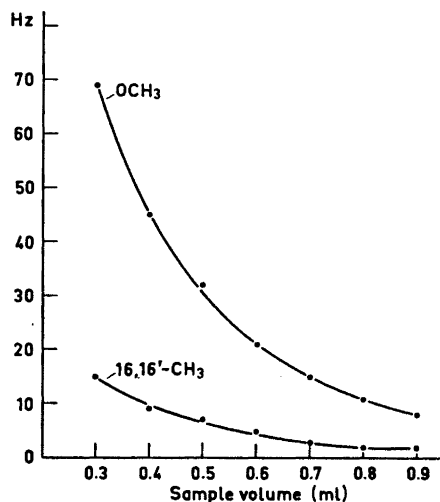
Fig. 2. Induced shifts of the methoxy and 16,16′-methyl signals of zeaxanthin dimethyl ether (7) at constant reagent/substrate ratio (1.0) on dilution of the sample volume. Reference: Unshifted signals.

tertiary alcohol rhodopin (3) with increasing concentrations of reagent up to a molar ratio of 4. The plot indicates that constant shift values are not reached until a molar reagent/substrate ratio of about 6. The fact that this ratio is considerably higher than those reported for other classes of compounds may be explained by the relative low substrate concentration of carotenoid NMR solutions. The concentrations employed are dictated by solubility.

At constant reagent/substrate ratios the induced shifts showed a nonlinear concentration dependency, decreasing with the absolute concentration of

reagent plus substrate. Fig. 2 illustrates the induced shifts of the methoxy and 16,16'-methyl signals of zeaxanthin dimethyl ether (7) plotted against the sample volume. These results are consistent with earlier observations [4],[15] and agree well with the assumption of a rapidly equilibrating collision complex according to eqn. 1.[2],[6]

$$\text{Metal(dpm)}_3 + \text{Substrate} \; \underset{}{\overset{K}{\rightleftharpoons}} \; \text{Metal(dpm)}_3.\text{Substrate} \tag{1}$$

The influence of temperature was not studied in the present work but the reported inverse relationship of the induced shifts with temperature [8] is in agreement with the pseudocontact term as given by Briggs et al.[13]

The inverse cube relationship of the induced shifts with the intramolecular metal-hydrogen distance, has been clearly demonstrated by Hinckley,[1] and it has been stated that angular variations play an important role.[7],[13] In the spectra of an in-chain substituted keto carotenoid (11),[35] a number of signals move slightly upfield on increasing concentrations of Eu(dpm)$_3$, whereas other signals move downfield as expected (Fig. 3). This allows some speculation as to
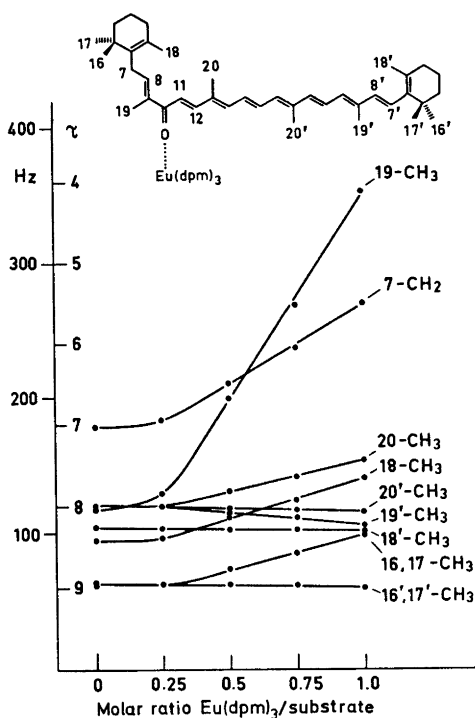

Fig. 3. Chemical shifts (Hz) of various signals of the keto carotenoid 11 with increasing concentrations of Eu(dpm)$_3$ at constant volume. Reference: TMS.

the conformation of the coordinated molecule. The results may be explained by assuming s-cis configuration around the 9-single bond resulting in small angular variations for one part and large variations for the other part of the molecule, as depicted in Fig. 3.
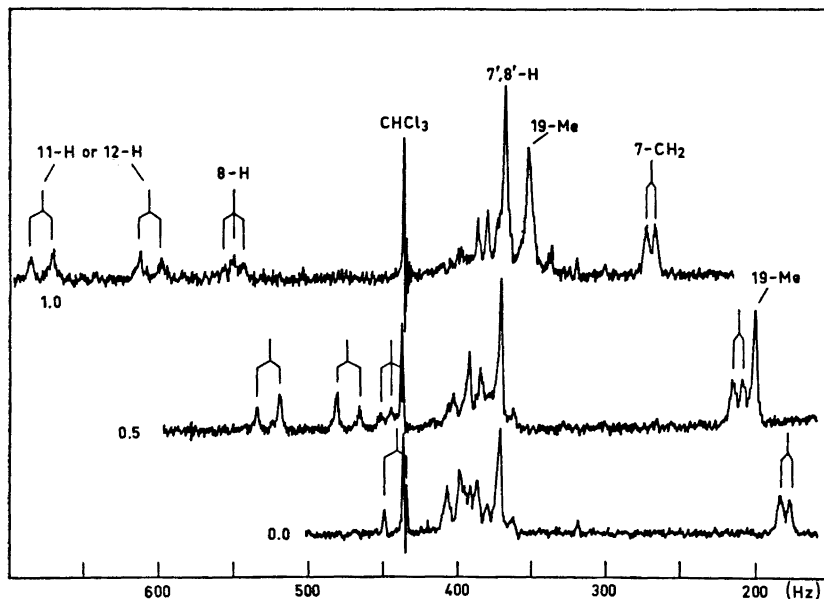
Fig. 4. Olefinic region of the keto carotenoid *11* at 0.0, 0.5, and 1.0 molar ratios of Eu(dpm)₃/*11*.

The keto carotenoid *(11)* also reveals an interesting feature of the olefinic region upon addition of Eu(dpm)₃, Fig. 4. The C-11 and C-12 olefinic protons give rise to two AB doublets with *trans* ($J = 16$ Hz) coupling; one of the doublets is hidden in the olefinic envelope. Both doublets are moved strongly downfield and out of the olefinic region upon addition of Eu(dpm)₃. Moreover, a triplet ($J = 7$ Hz) caused by the C-8 proton coupled with the C-7 methylene protons is also moved out of the olefinic region. These features, together with the strongly displaced C-19 methyl signal, completely define the partial structure around the complexing function, information which is not directly evident from the original spectrum.

The order of relative complexing power of functional groups has been stated to be amine, hydroxyl, carbonyl, ethers, esters, and nitrile.[6] Deviations have been reported.[12] The present study indicates that this also is the order for carotenoids judged by indirect comparison of the induced shift values obtained. Due to solubility problems, direct comparison of the results could not be made. The relative shifts (Table 1) for zeaxanthin *(5)*, zeaxanthin diacetate *(6)*, zeaxanthin dimethyl ether *(7)*, and rhodoxanthin *(8)* support this sequence of complexing power.

Steric hindrance seems to be of importance.[6] In our experiments the induced shifts of the methoxy signal of the tertiary aliphatic ether spirilloxanthin *(4)* are larger than those observed for zeaxanthin dimethyl ether *(7)*. Steric conflict between the ligands of the reagent and the substituted cyclohexene

Table 1. Relative shifts of the methyl signals of the carotenoids investigated relative to 16-Me.

Carotenoid	Methyl signals										Experimental conditions		
	16	17	18	19	20	20′	19′	18′	17′	16′	Substrate μmol	Molar ratio reag./substr.	Sample vol. ml
Rhodopin (3)	100	100	7	0.4	0	0	0	0	0	0	11	4.0	0.4
Spirilloxanthin (4)	100	100	10	0	0	0	0	10	100	100	20	2.0	0.8
Zeaxanthin (5)	100	92	64	26	8	8	26	64	92	100	20	2.0	0.8
Zeaxanthin diacetate (6)	100	68	38	6	0	0	6	38	68	100	20	2.0	0.3
Zeaxanthin dimethyl ether (7)	100	46	46	15	4	4	15	46	46	100	20	1.5	0.3
Rhodoxanthin (8)	100	100	71	28	8	8	28	71	100	100	20	2.0	0.3
Isozeaxanthin (9)	100	87	231	39	9	9	39	231	87	100	20	1.0	0.3
Canthaxanthin (10)	100	100	355	45	11	11	45	355	100	100	20	2.0	0.3
Keto carotenoid 11	100	100	125	608	89	−14	−41	−8	−11	−11	20	1.0	0.3
Crocetindial (12)[a]	−	−	−	48	8	8	48	−	−	−	20	2.0	0.4

[a] Relative to the aldehyde (H−8) signal.

ring may make coordination more difficult in the latter case. The difference in shifting power of lone pair functions may therefore be caused both by differences in basicity and steric hindrance, either causing different equilibrium constants or giving different metal-function distances. Both cases will result in changes in the average distance between metal and complexing function.

The magnitude of the induced shifts observed in our experiments are much smaller than those reported for other compounds. This may be explained by the relative dilute carotenoid NMR solutions.

It has been shown that the magnetic non-equivalence of protons α and even β to an asymmetric function is accentuated by addition of shift reagents, and allows geminal couplings to be measured.[5,6,15] The spectra of zeaxanthin dimethyl ether (7, Fig. 5) show the complex coupling pattern of the four pairs of protons at carbon atoms 2, 2′, 4 and 4′. Further identification of the individual protons should be possible using decoupling techniques.

Relative shifts have already been introduced for triterpenes [8] and it is our belief that relative shifts are the most reliable data for shift induced spectra. For a given shift reagent, relative shifts are characteristics of the substrate only and are not dependent on variations in temperature, relative and absolute concentrations as are the induced shifts. It is seen from Table 1 that there are considerable variations in the relative shifts of the methyl signals of the carotenoids investigated. The relative shifts are here measured in per cent of the more strongly shifted *gem.* methyl signal, since this is easily recognized. In cyclohexene derivatives with asymmetric centers the most shifted *gem.* methyl signal is accomodated with the 16-methyl rather than the 17-methyl group for the following reason: Assuming an equatorial functional group at the asymmetric center (absolute stereochemistry as shown [30]) and half-chair conformation of the cyclohexene ring, the cyclic compounds should have the shorter distance between the 16-methyl group and the complexing function.
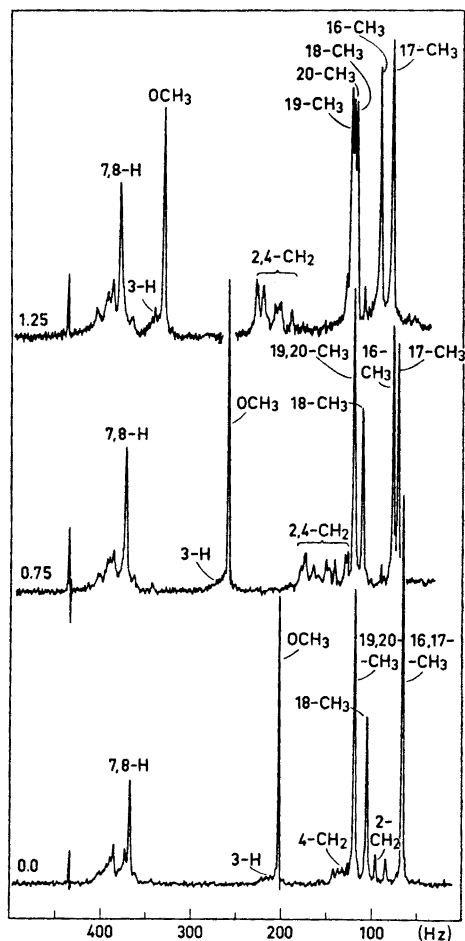
Fig. 5. NMR spectra of zeaxanthin dimethyl ether (7) at 0.0, 0.75, and 1.25 molar ratios of Eu(dpm)₃/7.

It is of interest to note how far along the polyene chain the effect of the shift reagent is observed (Table 1). In the case of rhodopin (3), it is noticeable at C-19, whereas for spirilloxanthin (4) only at C-18. For zeaxanthin diacetate (6), the effect is noticeable at C-19. For canthaxanthin (10), isozeaxanthin (9), rhodoxanthin (8), zeaxanthin (5), and zeaxanthin dimethyl ether (7) it is noticeable at C-20. In view of the results obtained for the keto carotenoid (11), one would expect the effect to be noticeable at least to the center of the carotenoid molecule. The effect of each function in symmetrically disubstituted carotenoids is additive; hence the region in which the shift reagent exerts an influence cannot be directly determined. The location of the complexing functions are in all cases reflected by the size of the relative shifts observed for each compound.

CONCLUSION

Europium shift reagents seem to offer great advantages in carotenoid NMR spectrometry. The use of these reagents to shift diagnostically useful signals out of their normal envelopes offers great advantages in locating functional groups.

Various in-chain substituted carotenoids are reported to possess *cis* bonds near to the oxygen substituent.[36,37] The position of the oxygen function should in such cases be easily recognized by this technique due to angular variations provided the conformation of the substrate is not changed by coordination.

Configurational studies may be facilitated using shift reagents, especially in locating *cis* double bonds in the polyene chain (*e.g.* diatoxanthin [38]) where the angular variations should be considerable.

It appears that only monofunctional and symmetrical bifunctional carotenoids may be readily analysed by this method. With more oxygen functions, identification of the signals in the shift induced spectra becomes unreliable even when using stepwise addition of the shift reagent. However, polyfunctional carotenoids may be analysed with this technique by using suitable model compounds and treating the results in an empirical manner. We have, in fact, employed this method in the structural elucidation of peridinin ($C_{39}H_{50}O_7$), by comparing the results with those of the structurally similar carotenoid fucoxanthin.[39]

EXPERIMENTAL

Dipivaloylmethane was synthesized from phenyl pivalate and pinacolone according to Man *et al.*,[40] and tris(dipivalomethanato)europium(III) according to Eisentraut and Sievers.[41]

Zeaxanthin was of natural origin (maize). The other carotenoids used were crystalline synthetic samples.

The NMR spectra were recorded on a Varian A-60A spectrometer. Deuteriochloroform (Merck) stored over colloidal silver was used as solvent and TMS as internal standard.

The shift reagent was added as aliquots of a 0.025 M solution in carbon tetrachloride. After addition of reagent, the samples (20 μmol of substrate; *ca.* 10−12 mg carotenoid) were evaporated in a stream of pure, dry nitrogen and redissolved in a constant, known volume of $CDCl_3$. The spectra of each carotenoid were recorded without and with five to eight different concentrations of shift reagent. The effect of absolute reagent/substrate concentration was investigated for zeaxanthin dimethyl ether (*7*) and canthaxanthin (*10*).

The spectra were recorded at the ambient temperature (*ca.* 40°C) of the spectrometer.

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