580. *Studies in Nuclear Magnetic Resonance. Part I. Methyl Groups of Carotenoids and Related Compounds.*

By M. S. Barber, J. B. Davis, L. M. Jackman, and B. C. L. Weedon.

The methyl absorption bands in the nuclear magnetic resonance spectra of 64 polyenes are collated and discussed. The band positions afford a valuable indication of the molecular environments of the various methyl groups present.

The scarcity of many carotenoids greatly handicaps studies on their structure. Much valuable information concerning the chromophores can readily be obtained from light-absorption studies, especially in the visible and the infrared region. However, despite a number of micro-methods of degradation and analysis, many of them developed originally to deal with this class of compound,[1-3] the nature of the end groups is often difficult to determine. The advent of nuclear magnetic resonance spectroscopy as a routine technique suggested a new approach to these problems. Tests showed that satisfactory spectra could be obtained on as little as 10 mg. of pigment, and that this material could subsequently be recovered for other investigations. To develop and evaluate the method we have determined the nuclear magnetic resonance spectra of 64 compounds of known structure. This survey provides useful information concerning both the methyl groups and the olefinic protons encountered in carotenoids. In this paper we confine ourselves to the methyl groups. In other publications we shall illustrate the use of the new method for structural studies on both carotenoids and the C_{40} compounds which are believed to be their precursors in Nature.

The majority of the nuclear magnetic resonance spectra were determined at 40 Mc. (More recent work indicates that better separation of the bands is observed, and that less sample is required, at 56·4 Mc.) The positions of the methyl bands (expressed in τ-values, as defined by Tiers [4]) are given in the Table. The band areas were in good agreement with those expected from the number of the various types of methyl known to be present. All compounds were examined in solution (2—5%), tetramethylsilane being used as an internal reference. The choice of solvent was sometimes dictated by the poor solubility of many carotenoids, and occasionally hot saturated solutions had to be employed. The τ-values determined in deuterochloroform, chloroform, and carbon tetrachloride agreed within experimental error, but bands recorded in pyridine solution were shifted by *ca.* 0·08 p.p.m. to lower fields. Differences of this magnitude are not as a rule of much significance, but must be taken into account in interpreting small changes in spectra with such closely related compounds as are under consideration here.

It is now well established that, at room temperature, the freedom of rotation of a methyl group about its three-fold symmetry axis is such as to render equivalent the environments of the three protons. Thus, in the absence of spin–spin coupling with protons on neighbouring atoms, the protons on methyl groups give rise to a single sharp peak in a nuclear magnetic resonance spectrum.

Methyl groups in paraffinic hydrocarbons normally have bands at 9·10—9·15, whereas those attached to carbon–carbon double bonds in simple olefins absorb in the range 8·30—8·40.[5] Methyl groups in carotenoids exhibit many deviations from these positions, and the shifts afford a valuable indication of the molecular environments of the groups in question. In all natural carotenoids of established structure, methyl groups are situated either on oxygen atoms or on fully substituted carbon atoms; therefore no splitting of the

[1] Karrer and Jucker, "Carotinoide," Birkhäuser, Basle, 1948.
[2] Holyer and Weedon, *Chem. and Ind.*, 1955, 1219.
[3] Entschel, Eugster, and Karrer, *Helv. Chim. Acta*, 1956, **39**, 1263.
[4] Tiers, *J. Phys. Chem.*, 1958, **62**, 1151.
[5] Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon, London, 1959.

methyl bands due to spin–spin coupling with a $C_{(\alpha)}$-proton is encountered. The bands appear as single peaks which are readily distinguished from absorption due to methylene groups, if any; the latter generally give broad bands as the result of complex spin–spin coupling.

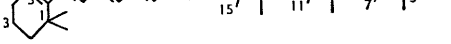
A common feature of the spectra of all carotenoids and related polyenes is a band in the region 7·95—8·15. From its relative intensity and its occurrence in the spectrum of methylbixin (12 *), which possesses no hydrocarbon end groups, this band has been ascribed to methyl groups attached to the non-terminal double bonds of the polyene chain. These " in-chain " methyls (I) are the most strongly de-shielded of all *C*-methyl groups in natural carotenoids. Their absorption bands are therefore easily recognised and provide a convenient means of distinguishing polyenes of the polyisoprenoid type from those without *C*-methyl groups (*e.g.*, corticrocin [6] and cortisalin [7]), or with methyl groups only at the end of the polyene chain (*e.g.*, degradation products of lagosin [8] and filipin [9]).

Only one " in-chain " methyl band is observed for β-carotene (25) (Fig. 1), lycopene (18) (Fig. 2), methylbixin (12), and many other carotenoids. Moreover the mono(" unhindered ")-*cis*-polyenes (8, 12, 36, 41, 43) which have been studied have a band at almost the same field as that of their " all-*trans* "-isomers (7, 11, 35, 40, 42). However, some gross modifications of the typical polyene chromophore cause small changes in the shielding of the " in-chain " methyls, and hence of the corresponding band positions; both crocetin dimethyl ester (10) and crocetindial (5), for example, have a peak at slightly lower fields (7·97) than that of the corresponding methyl groups in β-carotene (8·05). If the chain modification results in a difference in the shielding of the various " in-chain " methyls, then a broadening, or splitting, of the band is apparent. Thus the spectra of the acetylenes (4, 6, 27, 29, 30, 37, 39, 44, 46—49, 57, 59, 64) show that a methyl group (II) at a β-position with respect to the triple bond in a conjugated system gives rise to a separate band at slightly lower fields (7·87—7·95) than those of other " in-chain " methyls (Fig. 3). Differences in the shielding of the " in-chain " methyls are also evident in some polyenes with comparatively short, unsymmetrical, chromophores. The methyl esters of azafrin (cf. 58) and the related C_{27} acids (cf. 57 and 59), all have two " in-chain " methyl bands with relative intensities of 2 : 1, whilst apo-4-carotenal (50) has two of equal intensity. In vitamin A acetate (24) (Fig. 4) the methyl on the central double bond is of the " in-chain " type (I), whereas that on the terminal acyclic double bond resembles the methyl of isoprene ($\tau = 8{\cdot}17$).[5] This difference leads to a broad band at 8·05—8·10 which is clearly resolved into two peaks at 56·4 Mc. The methyl groups on the acyclic double bonds of the ionylidene-ethanols (32 and 33) also resemble that of isoprene more closely than those of the true " in-chain " type, and therefore give bands at comparatively high fields. The corresponding methyl in ethyl *trans*-ionylideneacetate (34) has a band at 7·71, as expected from the special steric relation of the *C*-methyl to the ester group.[5]

Methyl groups attached to the terminal carbon atoms of polyene chains, " end-of-chain " methyls (III), are less de-shielded than those which are " in-chain," and therefore give bands at higher fields. Thus the spectrum of lycopene (18) (Fig. 2) has a band at 8·18 (of half the intensity of that at 8·03), which may be assigned to the 5- and 5′-methyl groups.† A similar band is observed in the spectra of the hydrocarbons (16) and (17).

* Arabic numbers refer to compounds listed in the Table.

† The carbon atoms in carotenoid formulæ are numbered (cf. Karrer, *Bull. Soc. Chim. biol.*, 1948, **30**, 150), as illustrated below for β-carotene.



[6] Erdtman, *Acta Chem. Scand.*, 1948, **2**, 209.

[7] Gripenberg, *Acta Chem. Scand.*, 1952, **6**, 580.

[8] Dhar, Thaller, and Whiting, *Proc. Chem. Soc.*, 1958, 148; Dhar, Thaller, Whiting, Ryhage, Ställberg-Stenhagen, and Stenhagen, *ibid.*, 1959, 154.

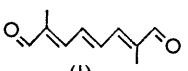
[9] Cf. Berkoz and Djerassi, *Proc. Chem. Soc.*, 1959, 316.

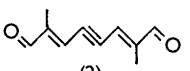
Compound no.[a]	Ref.	Solvent[d]	Paraffinic		Olefinic		
			gem-Me	Other paraffinic Me	Me on isolated or terminal C=C	Other olefinic Me[e]	Ester Me and some other bands
1	1, 2	CCl_4				8·06	0·52 (CHO)
2	1, 2	CCl_4				8·01 (J = *ca.* 1·0)	0·48 (CHO)
3	3	$CDCl_3$				8·00	0·39 ($J \sim 8$) (CHO)
4	3	$CDCl_3$				7·87	0·38 (J = 7·2) (CHO)
5	3	CCl_4				8·12, 7·97	—
		$CDCl_3$				8·10, 7·97	0·55 (CHO)
6	3	CCl_4				8·13, 7·88	—
7	1	$CDCl_3$				7·98	6·21
8	1	$CDCl_3$				7·98	6·20
9	1	$CDCl_3$				7·88 (J = 0·93)	6·26
10	4	$CDCl_3$				7·99	6·23
11	4	$CDCl_3$				8·03	6·24
12	Nat.	$CDCl_3$				8·05	6·23
13	Com.	CCl_4			8·37		7·93 (COMe
14	Nat.	CCl_4[g]			*ca.* 8·3 (complex)		
15	Nat.	CCl_4[g]			*ca.* 8·3 (complex)		
16	Exp.	$CDCl_3$[g]			8·20	8·08	
17	Exp.	$CDCl_3$[g]			8·18	8·04	
18	3	$CDCl_3$[g]			8·38, 8·31, 8·18	8·03	
19	5	$CDCl_3$		8·89 (J = 7·0)	8·44, 8·33	8·04	
20	6	CCl_4	8·85				7·91 (COMe)
21	6	CCl_4	8·88	*ca.* 8·93 (doublet)			7·94 (COMe)
22	Nat.	$CDCl_3$ / $CHCl_3$	8·83			8·02	7·70 (J = 6·8) (CH_2) 6·78 (OMe)
23	Com.	CCl_4[g]	8·96		8·33		7·92 (COMe)
24	Com.	CCl_4[g]	8·99		8·31	8·12, 8·07	8·02 (OAc)
25	Com.	CCl_4	8·98		8·31	8·05	
		$CHCl_3$	8·96		8·28	8·03	
		$CDCl_3$	8·97		8·28	8·03	
		C_5H_5N	8·89		8·21	7·96	
26	Nat.	$CDCl_3$[g]	8·92		8·26	8·05	
		C_5H_5N	8·85		8·17	7·98	
27	7	$CDCl_3$[g]	8·97		8·29	8·02, 7·88	
28	8, 9	CCl_4	8·81		8·21	8·04	
29	8, 9	CCl_4	8·81		8·21	7·99, 7·89	
30	8	CCl_4	8·99, 8·81		8·31, 8·20	8·02, 7·92	
31	10	$CDCl_3$	8·97		8·28	8·12(1), 8·02(4)	
32	11 *h*	CCl_4	9·05		8·36	8·22	
33	11 *h*	CCl_4	9·03		8·35	8·17	
34	11 *h*	CCl_4	9·00		8·33	7·71	
35	12	$CDCl_3$	8·98		8·29	8·12(1), 8·04(2)	6·28
36	12	$CDCl_3$	8·95		8·27	8·08(1), 8·01(2)	6·24
37	12	$CDCl_3$	8·98		8·30	8·03, 7·98, 7·90 †	6·28
38	12	$CDCl_3$	8·98		8·29	8·06	6·28

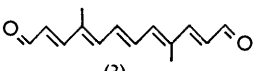
The " end-of-chain " methyls at positions 9 and 9′ in aurochrome (61), and the corresponding methyl groups in mutatochrome (62) and the C_{27} ester (59), give a band at even higher fields (8·26—8·28); a possible explanation of this difference is suggested below.

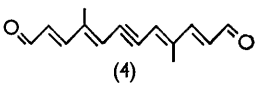
As shown by the spectra of the polyene aldehydes (1, 5, 6, 47, 49—51) and esters (7, 10, 38, 39, 42—46), " end-of-chain " methyl groups on a carbon atom which is α to a carbonyl group, are de-shielded by the latter and give a band at 7·98—8·13. This is normally

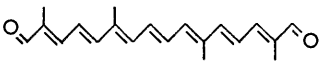
[10] Barber, Jackman, and Weedon, *Proc. Chem. Soc.*, 1959, 96.
[11] Braude, Jones, Koch, Richardson, Sondheimer, and Toogood, *J.*, 1949, 1890; Oroshnik, Karmas, and Mebane, *J. Amer. Chem. Soc.*, 1952, **74**, 295; Braude, Bruun, Weedon, and Woods, *J.*, 1952, 1419.
[12] Cf. Heilbron and Weedon, *Bull. Soc. chim.*, 1958, 83.

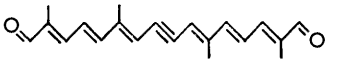

(1)

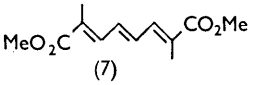

(2)

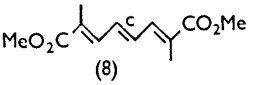

(3)

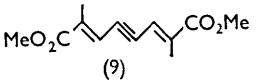

(4)

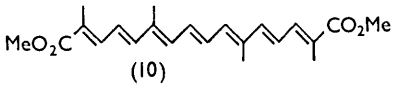

(5)

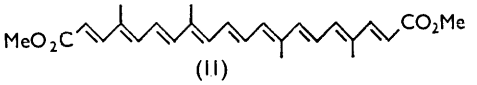

(6)


(7)

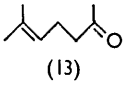

(8)

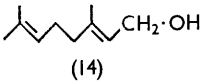

(9)

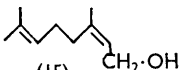

(10)

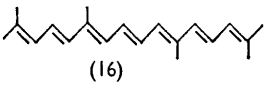

(11)

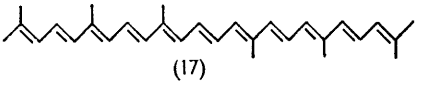

(12)

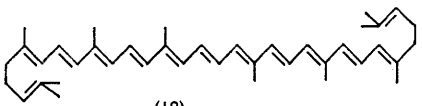

(13)

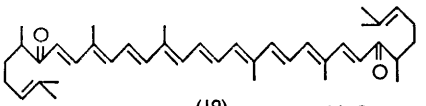

(14)

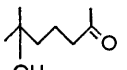

(15)

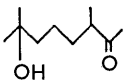

(16)

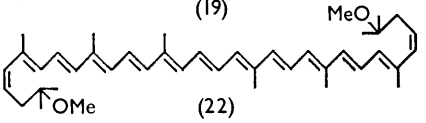

(17)

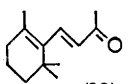

(18)

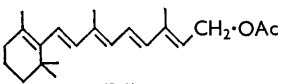

(19)

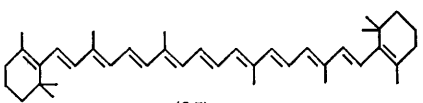

(20)

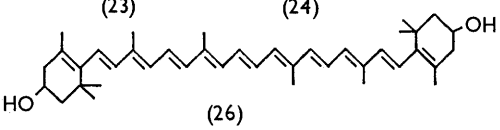

(21)

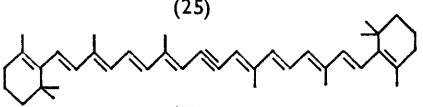

(22)

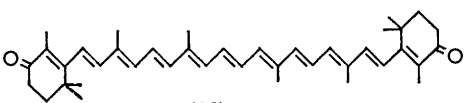

(23)

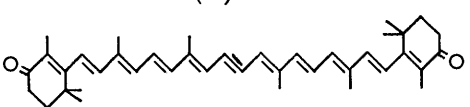

(24)

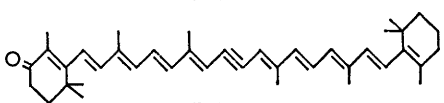

(25)

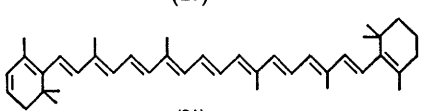

(26)

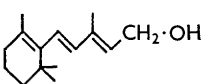

(27)

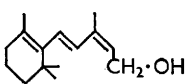

(28)

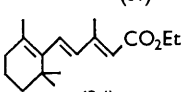

(29)

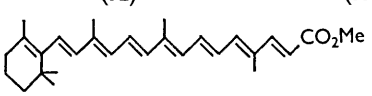

(30)

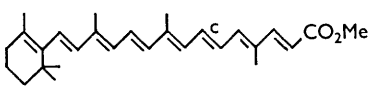

(31)


(32)

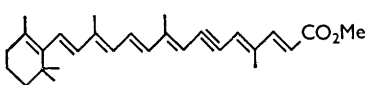

(33)

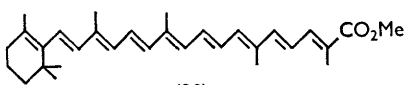

(34)




(36)

(35)


(37)


(38)

Compound no.[a]	Ref.[b]	Solvent[d]	Paraffinic: *gem*-Me	Paraffinic: Other paraffinic Me	Olefinic: Me on isolated or terminal C=C	Olefinic: Other olefinic Me[e]	Ester Me and some other bands
39	12	$CDCl_3$	8·97		8·28	8·02, 7·91	6·26
40	12	$CDCl_3$	9·00		8·29	8·07	6·28
41	12	$CDCl_3$	8·97		8·29	8·08	6·27
42	12	$CDCl_3$	8·97		8·28	8·03	6·26
43	12	$CDCl_3$	8·98		8·28	8·03	6·26
44	12	$CDCl_3$	8·97		8·29	8·02(3), 7·90(2)	6·23
45	12	$CDCl_3$[g]	8·99		8·30	8·06	6·28
46	12	$CDCl_3$	8·98		8·29	8·01(4), 7·90(2)	6·24
47	13	CCl_4	8·99		8·31	8·02(2), 7·87(1)	0·56 (CHO)
48	14	$CDCl_3$	8·98		8·29	8·02(1) 7·92(2)	0·41 (J = 7·2) (CHO)
49	14	CCl_4	8·98		8·31	8·13(1), 8·03(1), 7·90(2)	0·60 (CHO)
50	14	$CDCl_3$	8·96		8·27	8·13, 8·03, 7·99	0·55 (CHO)
51	14	$CDCl_3$[g]	8·96		8·24	8·10(1), 8·01(3)	—
52	15	$CDCl_3$	8·68			8·07, 8·03[i]	
53	16	$CDCl_3$	8·72			8·10, 8·02, 7·96	
54	Com.	CCl_4[g]	9·14, 9·08		8·29		7·83 (COMe)
55	Nat.	$CDCl_3$	9·15, 8·99, 8·94		8·36, 8·28	8·02	
56	17	$CDCl_3$	9·05			8·00(2)	
			8·86	8·86		7·85(1)	
57	17	$CDCl_3$	9·06			8·05(1)	6·25
			8·90[i]	8·86		7·93(2)	
58	Nat.	$CDCl_3$	9·16			8·07(1)	6·25
			8·87[i]	8·81		8·01(2)	
59	17	$CDCl_3$	8·89	8·58	8·26	7·95	6·22, 4·86
60	Exp.	$CDCl_3$	9·06			8·08	
			8·91[i]	8·87[i]			
61	Exp.	$CDCl_3$	8·90[k]	8·58	8·27	8·07	4·84[j]
62	Exp.	$CDCl_3$	8·96, 8·88	8·57	8·28	8·04	4·85
63	8	C_5H_5N	8·84 (J = 6·5)			8·02	
64	8	$CDCl_3$[g]	8·97, 8·86 (J = 6·8)		8·29	8·02, 7·88	

† Inflexion.

[a] A *trans*-configuration is assumed for all carbon–carbon double bonds unless marked c in the formula. [b] Reference to method of preparation; "Com." signifies a sample of commercial origin, "Nat." a natural carotenoid or its derivative, "Exp." that the preparation is described in the Experimental section. [d] Spectra were determined at 40 Mc. unless otherwise indicated. [e] Where more than one band is quoted, and these are of unequal intensity, the relative number of methyl groups is given in parentheses. [f] Band assignments are given in parentheses, except for ester-methyl (CO_2Me). [g] Spectrum determined at 56·4 Mc. [h] Prepared by W. Gee. [i] One of each pair of bands marked [i] must be due to a *gem*-methyl group; the assignment of the band at higher fields to this group is arbitrary. [j] Both protons attached to the heterocyclic ring(s). [k] For fine structure at 56·4 Mc., see text.

[1] Mildner and Weedon, *J.*, 1953, 3294. [2] Inhoffen, Isler, von der Bey, Raspé, Zeller, and Ahrens, *Annalen*, 1953, **580**, 7. [3] Isler, Gutmann, Lindlar, Montavon, Rüegg, Ryser, and Zeller, *Helv. Chim. Acta*, 1956, **39**, 463. [4] Isler, Gutmann, Montavon, Rüegg, Ryser, and Zeller, *ibid.*, 1957, **40**, 1242. [5] Barber and Weedon, unpublished work. [6] Warren and Weedon, *J.*, 1958, 3972. [7] Isler, Lindlar, Montavon, Rüegg, and Zeller, *Helv. Chim. Acta*, 1956, **39**, 249. [8] Akhtar and Weedon, *J.*, 1959, 4058. [9] Zeller, Bader, Lindlar, Montavon, Müller, Rüegg, Ryser, Saucy, Schaeren, Schwieter, Stricker, Tamm, Zürcher, and Isler, *Helv. Chim. Acta*, 1959, **42**, 841. [10] Isler, Lindlar, Montavon, Rüegg, and Zeller, *ibid.*, 1956, **39**, 274. [11] Huisman, Smit, van Leeuwen, and van Rij, *Rec. Trav. chim.*, 1956, **75**, 977; Robeson, Cawley, Weisler, Stern, Eddinger, and Chechak, *J. Amer. Chem. Soc.*, 1955, **77**, 4111. [12] Isler, Guex, Rüegg, Ryser, Saucy, Schwieter, Walter, and Winterstein, *Helv. Chim. Acta*, 1959, **42**, 864. [13] Rüegg, Lindlar, Montavon, Saucy, Schaeren, Schwieter, and Isler, *ibid.*, p. 847. [14] Rüegg, Montavon, Ryser, Saucy, Schwieter, and Isler, *ibid.*, p. 854. [15] Isler, Montavon, Rüegg, and Zeller, *ibid.*, 1956, **39**, 259. [16] Inhoffen and Raspé, *Annalen*, 1955, **594**, 165. [17] Akhtar and Weedon, unpublished results.

---

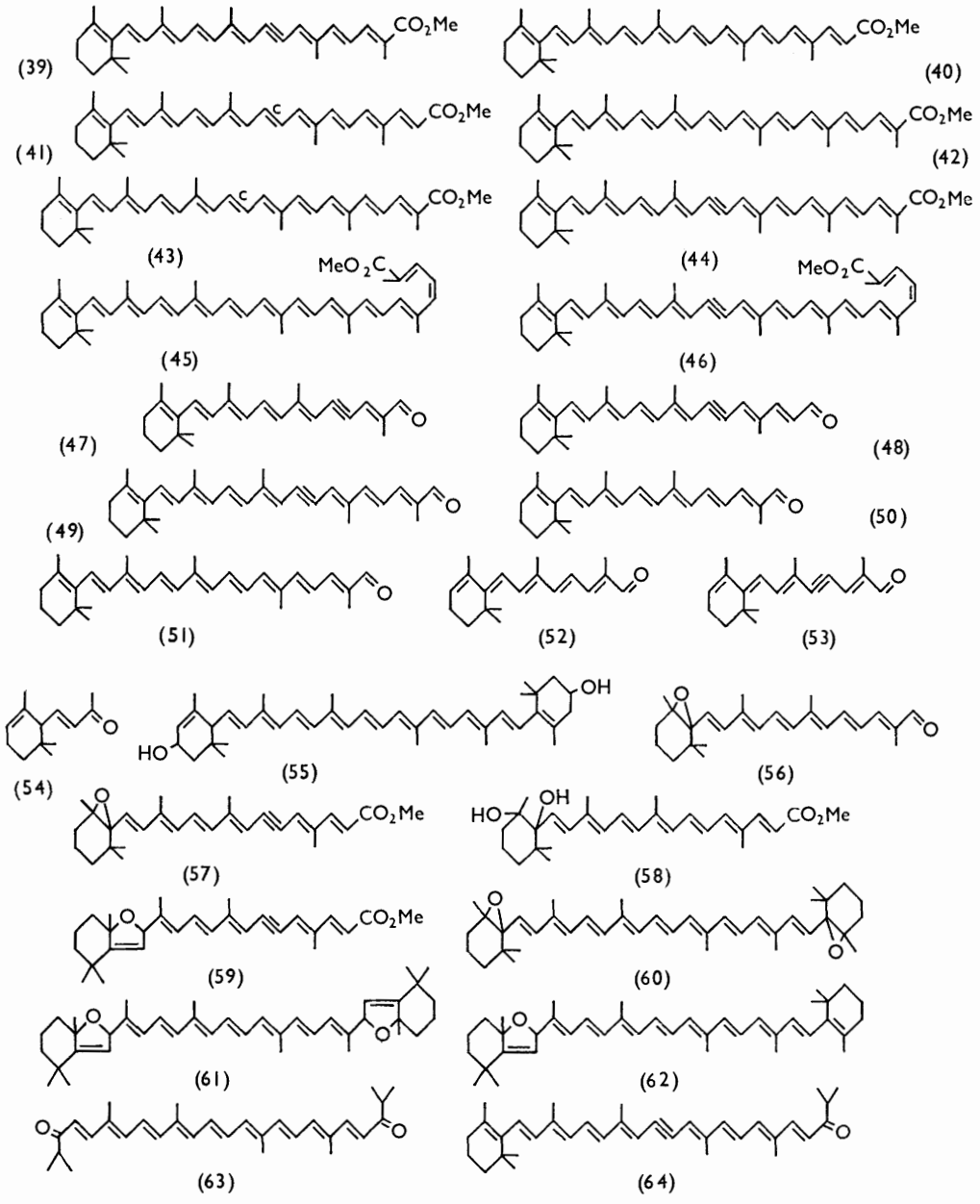
[13] Jackman and Lown, in the press.

[14] Akhtar and Weedon, unpublished results.

[15] Nazarov, Azerbaev, and Rakcheeva, *Bull. Acad. Sci. U.S.S.R.*, 1946, 419.

[16] Bolleter, Eiter, and Schmid, *Helv. Chim. Acta*, 1951, **34**, 186.

[17] Mildner and Weedon, *J.*, 1953, 3294; Inhoffen, Isler, von der Bey, Raspé, Zeller, and Ahrens, *Annalen*, 1953, **580**, 7.



resolved from the very similar " in-chain " methyl band in the spectra of the polyene aldehydes (Fig. 3), but not (at 40 Mc.) in those of the esters. An additional shift to lower fields is noticed with the acetylenic C_{10} dial (2) and the corresponding diester (9), owing to the influence of the triple bond; in both compounds the *C*-methyl band is split into a doublet ($J = ca.$ 1) by spin–spin coupling of the protons in each methyl group with a single olefinic proton. Splitting of this type is not normally encountered with the series of compounds under consideration, probably because coupling is seldom confined to one olefinic proton, and the fine structure of the methyl bands is not resolved. Moreover, the observed bands often represent two or more methyl groups in slightly different environments.

The various effects mentioned above must arise, at least in part, from long-range shielding associated with the diamagnetic anisotropies of ethylenic, acetylenic, and carbonyl bonds.[5] For instance, it is evident that the methyl protons in structures of type (II) lie over the triple bond, and hence in that region where the induced field is paramagnetic.[5]

We shall now consider the characteristic methyl absorptions of various end groups commonly encountered in carotenoids and related compounds. Lycopene (18) has bands

FIG. 1. *β-Carotene* (25) *in* CCl_4 *at* 56·4 Mc.

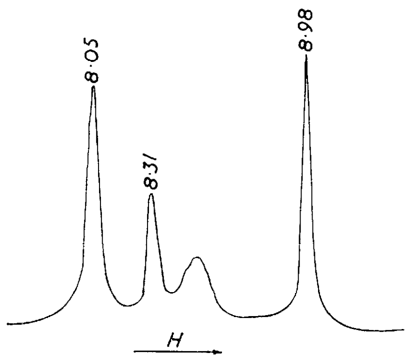


FIG. 2. *Lycopene* (18) *in* $CDCl_3$ *at* 56·4 Mc.

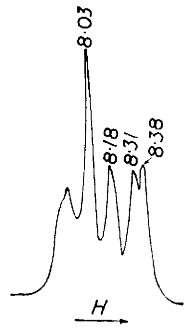


FIG. 3. 15,15′-*Dehydro-β-apo-8′-carotenal* (49) *in* CCl_4 *at* 56·4 Mc.

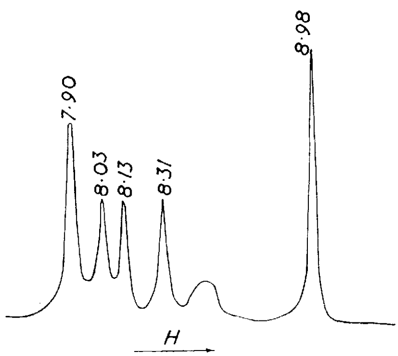
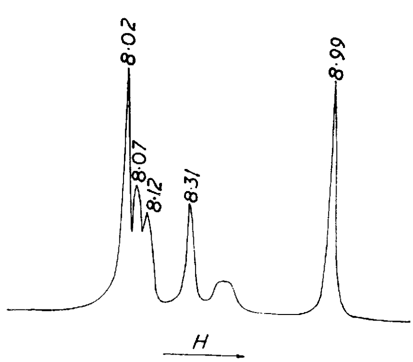
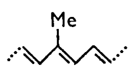


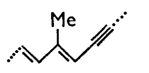
FIG. 4. *Vitamin A acetate* (24) *in* CCl_4 *a* 56·4 Mc.



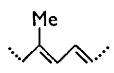
(Fig. 2) at 8·38 and 8·31 (each equal in intensity to that at 8·18) which must be attributed to the isopropylidene methyl groups. An explanation of the appearance of two bands is provided by a study of scale models. This shows that, during free rotation of the lycopene



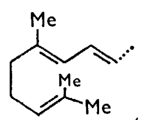
(I)



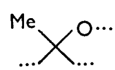
(II)



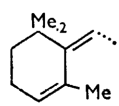
(III)



(IV)



(V)



(VII)

end groups, one methyl of each isopropylidene group approaches the polyene chain more closely than the other (cf. IV). The two methyl groups therefore experience different average fields and, in consequence, have different absorption bands. A similar situation is encountered with the related diketone (19) which has isopropylidene methyl bands at 8·44 and 8·33. Both geraniol (14) and nerol (15) have complex bands at *ca.* 8·3 since the 3-methyl group also absorbs in this region. The lower position (8·18) of the band due

to the corresponding 5- and 5′-methyl groups in lycopene clearly indicates the greater de-shielding effect of an extended polyene chain compared with that of an isolated double bond.

In contrast, the terminal *C*-methyl groups in the end groups of spirilloxanthin (22) are attached to a saturated carbon atom and absorb at 8·83. This position is somewhat lower than that observed with normal paraffinic methyls, and is similar to that found with t-butyl alcohol ($\tau = 8{\cdot}78$) and the two hydroxy-ketones (20) and (21). As pointed out elsewhere,[10] this paramagnetic shift is due to the oxygen substituent on the methyl-bearing carbon atom (cf. V).

In the synthetic ketones (19), (63), and (64), the methyl groups attached to tertiary carbon atoms give rise to doublets in the nuclear magnetic resonance spectra due to spin–spin coupling of the methyl-protons with the $C_{(\alpha)}$-proton. This is a feature which has not so far been encountered in the spectra of natural carotenoids.

The Table contains numerous examples showing that the trimethylcyclohexenyl (" β ") end group (VI) present in β-ionone (23), β-carotene (25), and many related compounds is associated with two methyl bands which occur near 8·30 and 8·97. These bands have a relative intensity of 1 : 2. That at 8·30 is obviously due to the olefinic 5-methyl group. Though this group is formally of the " end-of-chain " type, the band occurs at somewhat higher fields, and thus closely resembles those of the olefinic methyl groups in simple ethylenes. There is ample evidence from light-absorption data that, in compounds with β-end groups, steric hindrance between the *gem*-dimethyl group and the polyene chain forces the latter out of the plane of the cyclic double bond.[11] Conjugation between the cyclic double bond and the polyene chain is therefore restricted. Furthermore, it has been suggested that a carbon–carbon double bond can exert a long-range shielding effect, the sign and magnitude of which depend on the precise orientation of the proton in question with respect to the double bond.[5] In particular, it appears that a proton in a certain region above the plane of a double bond experiences positive shielding. Models indicate that protons of the olefinic methyl in a β-end group are influenced in this way by the 7,8-double bond, thus accounting for the shift to higher fields compared with the " end-of-chain " methyl in, for example, lycopene (18). A similar explanation, involving positive shielding by the 6,7-double bond, can be advanced to account for the position (8·26—8·28) of the " end-of-chain " methyl in the furanoid oxides (59, 61, 62) already mentioned.

The second (8·97) band associated with β-end groups must clearly represent both the *gem*-methyl groups. Evidently these groups are so orientated as to be de-shielded to a small degree by the neighbouring double bonds, but, because of a rapid interchange between two equivalent conformations of the cyclohexene ring, the two methyl groups experience identical environments, and hence only one band is observed.

A number of natural carotenoids have β-end groups hydroxylated at position 3. Substitution of this type has a negligible effect on the positions of the methyl bands, as demonstrated by the close similarity in the curves of β-carotene (25) and zeaxanthin (26). The hydroxy-substituent might be expected to favour a quasi-equatorial postion, thus avoiding a quasi-1,3-diaxial interaction with one of the *gem*-methyl groups. However, models indicate that the *gem*-methyl groups of the β-end group are similarly orientated with respect to the neighbouring double bonds in all conformations, and therefore no significant difference in shielding would be expected (cf. α- and epoxy-end groups below). Thus the observation of a single peak for the two *gem*-methyl groups is not unexpected.

The spectra of canthaxanthin (28) and 15,15′-dehydrocanthaxanthin (29) show that a 4-keto-group conjugated with the polyene chain shifts both methyl bands of the β-end group to slightly lower fields. That the effect is genuine is confirmed by the spectrum of 15,15′-dehydroechinenone (30) in which splitting of both methyl bands is clearly visible. The shift in the olefinic methyl band is akin to that observed with the " end-of-chain " methyl groups in the polyene aldehydes and esters mentioned above.

It is of interest to consider the effect of introducing a second, conjugated, double bond into the β-end group, although only two compounds possessing this feature are known in Nature (vitamin A_2 and retinene$_2$ [12]). The spectrum of 3,4-dehydro-β-carotene (31) differs from that of β-carotene (25) in two main respects: the intensity of the 8·30 band has been halved, and a band has appeared at 8·12 (clearly resolved at 56·4 Mc., but forming a shoulder on the 8·02 band at 40 Mc.) with a quarter of the intensity of the 8·02 band. The drop in intensity of the 8·30 band is to be expected since the " dehydro-β "-end-group contains no " end-of-chain " methyl [cf. spirilloxanthin (22) [10]]. The 5-methyl group in this dehydro-system must therefore be responsible for the new band at 8·12. Its difference from the other " in-chain " methyl groups may again be attributed to positive shielding by the 7,8-double bond.

The trimethylcyclohexenylidene, or " retro," end group (VII) * also lacks an " end-of-chain " methyl. From the two examples studied (52 and 53), it appears that the *gem*-methyl groups in retro-systems are, as expected, equivalent and absorb near 8·70, and that the 5-methyl group, which is intermediate in character between that of isoprene and a typical " in-chain " methyl, absorbs near 8·10. The acetylenic retro-aldehyde (53) exhibits three olefinic methyl bands of equal intensity. The band at 7·96 is broad, but unresolved, and its position indicates that it is due to the " in-chain " methyl group adjacent to the triple bond. The band at 8·02 is a sharp doublet similar in appearance and position to that observed with the C_{10} acetylenic dialdehyde (2) and diester (9), indicating that the methyl-protons are coupled with only one olefinic proton. Therefore the 8·02 band must be associated with the methyl group on the α-carbon atom to the aldehyde group. The absorption at 8·10 can be ascribed to the 5-methyl of the retro-system. The band is broad because the methyl-protons are coupled, not only with the olefinic proton at $C_{(4)}$, but also with the 3-methylene protons (cf. ref. 13).

Another well-known end group, which is occasionally found in natural compounds, is that illustrated by α-ionone (54). The latter's spectrum shows that the " α-end group " gives rise to three methyl bands of equal intensity. One band occurs at *ca.* 8·30, and is clearly due to the olefinic methyl. The others occur at high fields, as would be expected for methyl groups in essentially saturated environments, and must represent the two *gem*-methyl groups which, unlike those in the β-, dehydro-β-, and retro-end groups, are no longer equivalent. This result is not surprising since the end group would doubtless favour the conformation (VIII), in which the bulky side-chain is quasi-equatorial. Models reveal that, in contrast to the situation with the hydroxy-β-end groups discussed above, there is a marked difference in the spatial relation of the two *gem*-methyl groups to the neighbouring double bonds. In particular, one of them may be positively shielded by the cyclic double bond. The spectrum of lutein (55) is compatible with the presence of (hydroxylated) end groups of both α- and β-type.

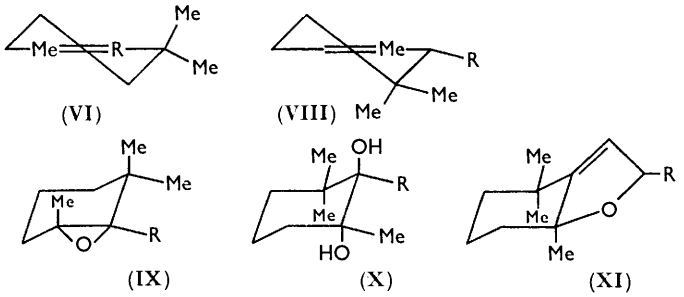
End groups of two other important classes of natural carotenoid, the epoxides and furanoid oxides, have still to be considered. The spectrum of β-carotene diepoxide (60), and that of the C_{27} epoxy-ester (57), show that the epoxide end group (IX) also contains three non-equivalent methyl groups. A band at *ca.* 8·86 may reasonably be assigned to the 5-methyl group; the de-shielding is comparable with that of the 1-methyl groups in spirilloxanthin, and may again be ascribed to the influence of the $C_{(\alpha)}$-oxygen substituent. The two *gem*-methyl groups are evidently influenced to different extents in the preferred conformation of the end group, presumably because one of them bears a *cis*-relation to the polyene chain, and hence give rise to separate bands at *ca.* 9·06 and 8·90. The latter position is very similar to that of the 5-methyl group, and it is noteworthy that the two bands were not resolved (at 40 Mc.) in the spectrum of the epoxy-aldehyde (56).

Methyl azafrin (58) has a related dihydroxy-system which is believed to favour the

* Systems of type (VII) were first designated " retro " by Oroshnik *et al.* (*J. Amer. Chem. Soc.*, 1952, **74**, 295). They have since been termed " dehydro-retro " by Isler *et al.* (*Helv. Chim. Acta*, 1956, **39**, 259).

conformation (X).[14] Its spectrum also shows three bands attributable to the end-group methyls. One of these may be positively shielded by the polyene chain since it has a band as high as 9·16.

Three furanoid oxides have been examined. At 56·4 Mc. the spectrum of aurochrome



(61) showed bands at 8·89, 8·84, and 8·74 with relative intensities of *ca.* 6 : 5 : 1. Since absorption at 8·74 with carotenoids must be due to methyl groups, the relative intensity of this band leads us to the conclusion that our synthetic specimen of aurochrome is a mixture of epimers (this is not unexpected from the mode of preparation), and that the three bands mentioned above all arise from the *gem*-methyl groups. The 5- and the 5′-methyl group are strongly influenced by both the oxygen atom [cf. 1-methyl group in spirilloxanthin (22)] and the double bond of the furanoid ring, and give a peak at 8·56 which shows some evidence of multiplicity. This may again indicate that the specimen is a mixture of epimers, but may also be due to the fact that the absorption overlaps that of methylene-protons. In the carotenoid field, methyl absorptions are rare in the 8·56 region, though one or more *broad* bands due to methylene groups are frequently observed.

A striking feature of the aurochrome spectrum is a band at 4·84 with an intensity equivalent to four protons. This is assigned to the protons attached to the two heterocyclic rings. The resonances of the two types of proton are accidentally degenerate, and therefore no spin–spin coupling is observed.

At 56·4 Mc. the spectrum of mutatochrome (62) shows the characteristic absorption of both the aurochrome and β-carotene end groups, as expected. At 40 Mc. the spectra of aurochrome, mutatochrome, and the C_{27} ester (59) are clearly recognisable as those of furanoid oxides, though the complexity of the bands due to the end-group methyls is not resolved.

We have sought to demonstrate that nuclear magnetic resonance spectra can aid greatly in the recognition of end groups, and other structural features, in carotenoids and related polyenes. It must, however, be emphasised that some of the very small changes in band positions to which we have drawn attention in this survey of known compounds, would be difficult to recognise with certainty in the spectrum of an unknown compound. Fine differences in band positions must therefore be interpreted with caution, and only in conjunction with other physical and chemical evidence.

Experimental

Visible-light absorption data for pure compounds were determined in benzene.

M. p.s determined on a Kofler block are corrected.

The nuclear magnetic resonance spectra were determined with a Varian 4300 spectrometer with a 40 or 56·4 Mc. oscillator. The measurements were made in dilute solutions (2—5%) and tetramethylsilane was used as an internal reference. The spectra were calibrated by the usual side-band technique and the results given in the Table are the averages of at least three separate determinations. A careful study of the reproducibility of line position was made for β-carotene in deuterochloroform, carbon tetrachloride, and pyridine; the standard deviations of the mean were respectively 0·005, 0·006, and 0·005 p.p.m.

2,2-*Dimethylallyltriphenylphosphonium Bromide*.—Reduction of methyl senecioate (22·8 g.)

with lithium aluminium hydride (5 g.) in ether (230 c.c.) at 20° led to 2,2-dimethylallyl alcohol (12 g., 70%), b. p. 70°/40 mm., 140°/756 mm., n_D^{22} 1·4410 (Nazarov *et al.*[15] give b. p. 140—142°, n_D^{20} 1·4410).

The alcohol (11 g.) in light petroleum (b. p. 40—60°) (25 c.c.) was added during 30 min. to a cooled (−25° to −30°) solution of phosphorus tribromide (12·5 g.) and pyridine (1·9 g.) in the same solvent (30 c.c.).[16] The mixture was stirred for 18 hr. and the temperature allowed to rise gradually to 20°. Distillation gave 2,2-dimethylallyl bromide (12·5 g., 66%), b. p. 70°/90 mm., n_D^{20} 1·4865 (lit.,[16] b. p. 77—78·5°/135 mm.).

The bromide (12·5 g.) was added to a solution of triphenylphosphine (25 g.) in benzene (25 c.c.). After 20 hr. the solid (31·5 g., 90%), m. p. 228—231°, which had separated was collected, washed with benzene and light petroleum (b. p. 40—60°), and dried *in vacuo*. Recrystallisation of a specimen from water gave the *phosphonium bromide* as cubes, m. p. 237—238° (Kofler block) (Found: Br, 19·3. $C_{23}H_{24}PBr$ requires Br, 19·4%).

2,6,11,15-*Tetramethylhexadeca*-2,4,6,8,10,12,14-*heptaene*.—0·6N-Ethereal phenyl-lithium (35 c.c.) was added rapidly to a stirred suspension of the above phosphonium salt (10·3 g.) in ether (100 c.c.). After 30 min., a solution of 2,7-dimethylocta-2,4,6-triene-1,8-dial [17] (656 mg.) in methylene chloride (30 c.c.) was added during 5 min. The mixture was refluxed for 5 hr., diluted with methanol (100 c.c.), and then kept at 0° overnight. The crystals (476 mg.), m. p. 158—161°, were collected. Recrystallisation from chloroform–methanol gave the *hydrocarbon* as yellow needles, m. p. 166° (Kofler block) (Found: C, 89·2; H, 10·6. $C_{20}H_{28}$ requires C, 89·5; H, 10·5%), $\lambda_{max.}$ 432, 407, and 385 mμ ($10^{-3}\varepsilon$ 135, 132, and 77 respectively). The infra-red light absorption spectrum showed no bands attributable to oxygen functions.

2,6,10,15,19,23-*Hexamethyltetracosa*-2,4,6,8,10,12,14,16,18,20,22-*undecaene*.—Conversion of the above phosphonium bromide (5·2 g.) into the phosphorane, and reaction with crocetindial [18] (592 mg.) in methylene chloride (40 c.c.), in the manner described in the preceding experiment, gave a brown solid. Crystallisation from chloroform–methanol, or from a large volume of benzene, gave the *hydrocarbon* (400 mg.) as red needles, m. p. 210—212° (decomp.) (Kofler block) (Found: C, 89·65; H, 10·4. $C_{30}H_{40}$ requires C, 89·95; H, 10·05%), $\lambda_{max.}$ 518, 483, and 452 mμ ($10^{-3}\varepsilon$ 124, 146, and 102 respectively). The infrared light absorption spectrum showed no bands attributable to oxygen functions.

β-*Carotene Diepoxide*.—The method of Karrer and Jucker [19] was modified in the following way. Ethereal monoperphthalic acid (*ca.* 0·5N; 2 equiv., 4 atom-equiv. of oxygen) was added to a solution of β-carotene (187 mg.) in ether (400 c.c.) ($\lambda_{max.}$ 479 and 451 mμ), and the mixture was kept at 20° until there was no further shift in the wavelength of maximal absorption (24 hr.). The solution ($\lambda_{max.}$ 468 and 439 mμ) was washed thoroughly with saturated sodium hydrogen carbonate, dried (Na_2SO_4), and evaporated under reduced pressure. Crystallisation from benzene–methanol gave the diepoxide (89 mg.), m. p. 184—185° (Kofler block), 188—189° (evacuated capillary; uncorr.), $\lambda_{max.}$ 481, 451, and 426 mμ ($10^{-3}\varepsilon$ 127, 136, and 90 respectively) [Karrer and Jucker [19] give m. p. 184° (evacuated capillary, uncorr.), $\lambda_{max.}$ 485 and 456 mμ]. The specimen used to determine the nuclear magnetic resonance spectrum was recovered; its visible-light absorption properties were unchanged.

When a greater excess of per-acid was used, the epoxide was partly rearranged into the furanoid oxide.

Mutatochrome and Aurochrome.[19]—Two drops of chloroform saturated with hydrogen chloride were added to a solution of crude β-carotene diepoxide (173 mg.) in chloroform (150 c.c.). After 4 min., the solution was washed with saturated sodium hydrogen carbonate, dried (Na_2SO_4), and evaporated. Chromatography on alumina (Grade III—IV [20]) from 3 : 7 benzene–light petroleum (b. p. 60—80°) gave 3 main bands. In order of elution these gave: (i) β-Carotene (*ca.* 5 mg.); (ii) mutatochrome (45 mg.) which crystallised from benzene–methanol in orange leaflets, m. p. 159—160° (Kofler block), 161—162° (evacuated capillary; uncorr.), $\lambda_{max.}$ 463, 437, and 416 (inflexion) mμ ($10^{-3}\varepsilon$ 101, 113, and 77 respectively) [Karrer and Jucker [19] give m. p. 163—164° (evacuated capillary, uncorr.), $\lambda_{max.}$ 470 and 440 mμ]; (iii) aurochrome (45 mg.) which crystallised from benzene–methanol in golden leaflets, m. p. 187—189° (Kofler), 195—197° (evacuated capillary, uncorr.), $\lambda_{max.}$ 434, 409, and 387 mμ ($10^{-3}\varepsilon$ 115, 116, and 73·5 respectively) [Karrer and Jucker [19] give m. p. 185° (evacuated capillary, uncorr.), $\lambda_{max.}$ 440 mμ].

[18] Isler, Gutmann, Lindlar, Montavon, Rüegg, Ryser, and Zeller, *Helv. Chim. Acta*, 1956, **39**, 463.
[19] Karrer and Jucker, *Helv. Chim. Acta*, 1945, **28**, 427.
[20] Brockmann and Schodder, *Ber.*, 1941, **74**, 73.

The specimens of mutatochrome and aurochrome were recovered after the determination of their nuclear magnetic resonance spectra; their visible-light absorption properties were unchanged.

The authors are greatly indebted to Hoffmann-La Roche Ltd. (Basle) and Roche Products Ltd. (Welwyn Garden City) for generous gifts of many chemicals and for financial support (to J. B. D.). One of them (M. S. B.) thanks the Department of Scientific and Industrial Research for a research studentship. They also thank Professor R. Kuhn for the specimen of methyl azafrin. Analyses were carried out in the microanalytical laboratory (Miss J. Cuckney) of this Department.

DEPARTMENT OF CHEMISTRY, IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY,
SOUTH KENSINGTON, LONDON, S.W.7. [*Received, December 8th*, 1959.]