316. *Studies in Nuclear Magnetic Resonance. Part IV.*[1] *Stereochemistry of the Bixins.*

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By means of nuclear magnetic resonance, methyl natural bixin is shown to be the *cis*-4-, neomethylbixin A the *cis*-8-, and neomethylbixin C either the *cis*-4,*cis*-8- or the *cis*-4,*cis*-12-isomer.

Natural bixin is shown to have the *cis*-16-structure.

BIXIN (I; R = H) was the first carotenoid in which geometrical isomerism was encountered.[2-6] The common natural isomer of this half-ester, which is the principal pigment in seeds of *Bixa orellana*, gives a higher-melting " stable " form on treatment with catalytic amounts of iodine.[3,4] In an attempt to examine the stereochemical difference between the two forms, Karrer and Solmssen[6] degraded both by carefully controlled permanganate oxidation to apo-1- (II), apo-2- (III), and apo-3-norbixinal methyl ester (IV). The two bixins gave different crystalline apo-1-esters. The apo-2-esters were also thought to be different, but a decision was made difficult by the failure of the products to crystallise except as their derivatives.[7] However, the two bixins were

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[1] Part III, Jackman and Wiley, *J.*, 1960, 2886.
[2] Herzig and Faltis, *Annalen*, 1923, **431**, 40.
[3] Karrer, Helfenstein, Widmer, and van Itallie, *Helv. Chim. Acta*, 1929, **12**, 741.
[4] Kuhn and Winterstein, *Ber.*, 1932, **65**, 646; 1933, **66**, 209.
[5] Zechmeister, *Chem. Rev.*, 1944, **34**, 267.
[6] Karrer and Solmssen, *Helv. Chim. Acta*, 1937, **20**, 1396.
[7] Karrer and Jucker, " Carotinoide," Birkhäuser, Basle, 1948,

reported to give the same apo-3-ester, which was obtained crystalline from some preparations. Karrer and Solmssen, therefore, concluded that the isomeric bixins probably differ in geometrical configuration about the third double bond from the carboxyl group, though in view of the uncertainty about the distinction between the two apo-2-esters the possibility that the second double bond was the site of stereochemical difference could not be entirely excluded.[6,7] (It does not seem to have been considered at this stage that more than one of the first three double bonds might be involved.) Later, visible- and ultraviolet-light absorption studies by Zechmeister and Escue[5,8] on the related diesters (I; R = Me) showed that methyl " stable " bixin had the " all-*trans* "-configuration, and methyl natural bixin * a mono-unhindered *cis*-structure, thus excluding Karrer and Solmssen's *cis*-6-formulation † for bixin A *cis*-4-structure for methyl natural bixin was favoured by Zechmeister and Escue, but subsequent infrared light absorption studies by Lunde and Zechmeister[9] led to the suggestion of a *cis*-2-formulation The " central-*cis* " configuration is excluded by the infrared[9] and other evidence;[10] moreover this isomer has now been synthesised by an unambiguous route and shown to be different from methyl natural bixin.[9,11]

It is obvious that there are as yet no satisfactory answers to the two main problems concerning stereochemistry in the bixin series, *viz.*, (i) the location of the *cis*-bond in methyl natural bixin, and (ii) the position of the *cis*-bond with respect to the free carboxyl group in bixin itself. Previous studies on nuclear magnetic resonance spectroscopy have shown that both chemical shifts and spin–spin coupling constants can afford precise information about the stereochemistry of ethylenic double bonds.[1,12] The solution of the above problems by these means is now reported.‡

The spectra of methyl " all-*trans* "-bixin and methyl natural bixin have many features in common, *viz.*, bands due to the *O*-methyl (τ 6·24) and *C*-methyl protons (8·03), a broad band (near 3·45) in the olefinic-proton region, and a doublet at 4·12 (*J* 15·8 c./sec.) equivalent to two protons. However, the two spectra differ in the region below 3·0; that of methyl " all-*trans* "-bixin exhibits a doublet at 2·61 (*J* 15·8) equal in intensity to the doublet at 4·12, whereas that of methyl natural bixin has doublets at 2·63 (*J* 15·8) and 2·07 (*J* 15·8) each of which has half the intensity of the doublet at 4·12.

The doublets at 4·12 and 2·61 in the spectrum of methyl " all-*trans* "-bixin clearly arise from two interacting olefinic protons: the relative intensities of the components of the doublets are in good agreement with those calculated from the chemical shift and coupling constant; furthermore, the separation of the components of each doublet is the same at 56·4 as at 40 Mc./sec. The only isolated pairs of mutually interacting protons in methyl " all-*trans* "-bixin are those of the equivalent double bonds at the two ends of the polyene chain. By analogy with many $\alpha\beta$-ethylenic esters[12] the high- and the low-field doublets can be assigned to the protons α and β respectively to the methoxycarbonyl groups, and the observed coupling constant is in good agreement with those of other *trans*-olefins.[13]

The spectrum of methyl natural bixin shows that both the α-protons and one of the β-protons are equivalent to those found in the " all-*trans* "-isomer, whilst the remaining β-proton gives rise to a band which is shifted by 0·56 p.p.m. to lower fields. This shift cannot be associated with a change in stereochemistry of one of the terminal double bonds

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* This compound has also been called " natural methylbixin." For the previous nomenclature in the bixin series see refs. 7 and 8.

† Geneva numbering; CO_2H in bixin = 1.

‡ A summary of the main results was given in *Proc. Chem. Soc.*, 1960, 23.

[8] Zechmeister and Escue, *J. Amer. Chem. Soc.*, 1944, **66**, 322.

[9] Lunde and Zechmeister, *J. Amer. Chem. Soc.*, 1955, **77**, 1647.

[10] van Hassalt, *Rec. Trav. chim.*, 1911, **30**, 1; 1914, **33**, 192.

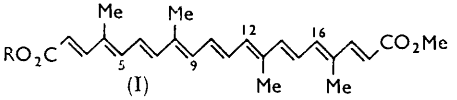
[11] Inhoffen and Raspé, *Annalen*, 1955, **592**, 214.

[12] Jackman and Wiley, *J.*, 1960, 2881.

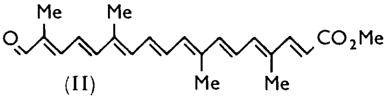
[13] Bishop and Richards, *Mol. Phys.*, 1960, **3**, 114.

because the coupling constants are unaltered and a value of 10—12 c./sec. would have been expected for a *cis*-bond.[13,14] The observed paramagnetic shift can only result from a change in configuration in the immediate neighbourhood of one terminal double bond since the appropriate absorptions of the esters[15] (VI) (4·13, 2·55; $J = 15·65$) and (VII) (4·18, 2·60; J 15·6) are virtually identical with those of their " all-*trans* "-isomers (4·19, 2·62, J 15·5; and 4·17, 2·60, J 15·5, respectively). It is therefore necessary to postulate that methyl natural bixin has the *cis*-4-structure, and that the shift is due to the proximity of the β-proton at the *cis*-end of the molecule to the 6,7-double-bond; there is evidence to suggest that the magnetic anisotropy of a carbon–carbon double bond deshields a proton situated close to it in the plane of the bond.[14]

To test the above hypotheses further a pair of model compounds,[16] (VIII) and (IX),



(I)



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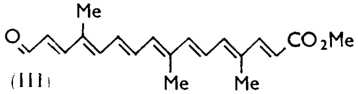
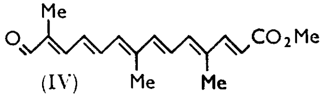


(III)



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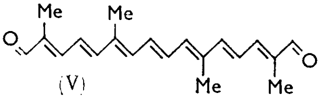
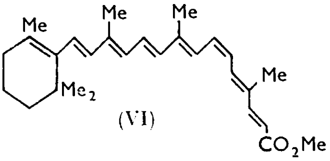
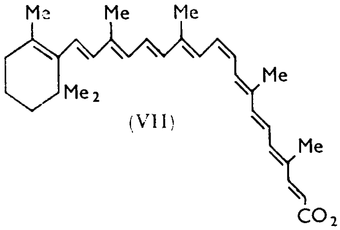


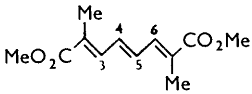
(V)



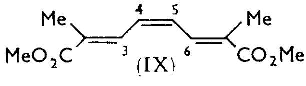
(VI)



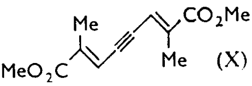
(VII)



(VIII)



(IX)



(X)

have been examined. In (IX) the β-protons bear the same relation to the 6,7-double bond as that just postulated for one of the β-protons in methyl natural bixin. An analysis (see below) of the complex region of the spectra of (VIII) and (IX) shows that the absorption due to the β-protons in the *cis*-isomer (IX) is shifted (0·58 p.p.m.) in a similar manner to that observed with one of the β-protons in methyl natural bixin. There is therefore no doubt that methyl natural bixin has the *cis*-4-structure.

By thermal or photochemical stereomutation of methyl " all-*trans* "-bixin and methyl natural bixin, Zechmeister and Escue[8] obtained two other crystalline isomers, neo A and neo C respectively, as the principal products. Light-absorption studies revealed that neo A also has a mono-unhindered *cis*-structure.[8,9] A strong " *cis*-peak " indicated that the *cis*-bond is near the centre of the molecule. Since neo A differs both from methyl natural bixin and from the synthetic " central-*cis* " -isomer,[9] it must have the *cis*-8-structure. The visible-light absorption properties of neo C are those of an isomer with two unhindered *cis*-bonds, and the infrared spectrum indicates the absence of a central *cis*-bond.[5,9] In the nuclear magnetic resonance spectrum the band pattern due to the α- and β-protons (4·13, 2·60, 2·03) is very similar to that observed with methyl natural bixin. Neo C must

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[14] Jackman, " Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon, London, 1959, p. 129.

[15] Isler, Guex, Rüegg, Ryser, Saucy, Schwieter, Walter, and Winterstein, *Helv. Chim. Acta*, 1959, **42**, 864.

[16] Mildner and Weedon, *J.*, 1953, 3294.

therefore be either the *cis*-4,*cis*-8- or the *cis*-4,*cis*-12-isomer, probably the latter since neo C is formed under conditions which convert methyl " all-*trans* "-bixin into the neo A isomer.

In an approach to the second main problem, the insolubility of the bixins in common solvents precluded direct comparison of their nuclear magnetic resonance spectra. A search for more suitable solvents was not undertaken because it was expected that the shielding contributions of the carboxy- and methoxycarbonyl groups would be so similar that no unambiguous assignment of the bands would be possible. Attention was therefore directed to the *cis*-apo-1-norbixinal methyl ester (II) described by Karrer and Solmssen;[6] a preliminary examination of crocetindial (V) revealed that the band due to the proton β to the aldehyde group in (II) would not be resolved from the broad band near 3·45. The spectrum of the *cis*-apo-1-ester in the olefinic proton region showed only two doublets (4·08, *J* 15·8; 2·04, *J* 15·8), each equivalent to one proton. Since these are clearly characteristic of the *cis*-end of methyl natural bixin, the *cis*-bond in natural bixin itself must be assigned to the 16-position in the esterified half of the molecule. This conclusion is not necessarily at variance with the degradative results of Karrer and Solmssen. Although it was stated that both natural and " all-*trans* "-bixin yielded the same apo-3-nor bixinal methyl ester (IV), the only experimental details given of the isolation of the latter from a compound of the *cis*-series relate to the oxidation of *methyl* natural bixin in

FIG. 1.* *The observed and theoretical spectra at* 40 *Mc./sec. of methyl " all-*trans *" - 2,7 - dimethylocta - 2,4,6-trienoate* (VIII). *Lines* 6 *and* 11 *of the B half-spectrum overlap the A half-spectrum. The band a arises from transitions of the A nuclei.*

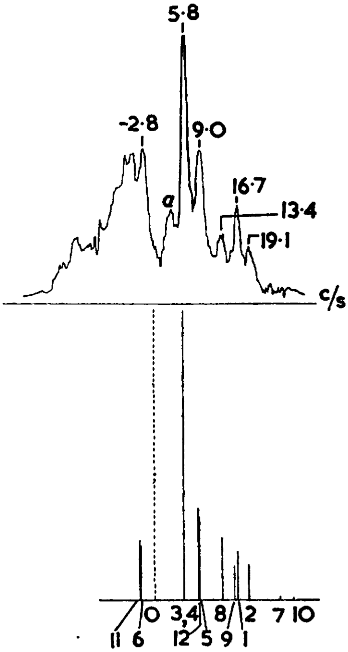
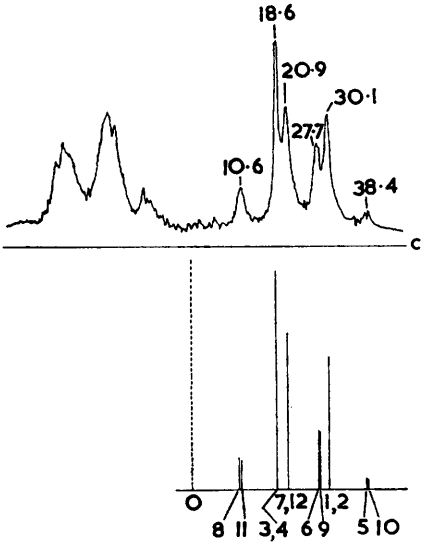


FIG. 2.* *The observed and theoretical spectra at* 40 *Mc./sec. of methyl 2,7-dimethylocta-*trans-2,cis-4,trans-6-*trienoate* (IX).



* The line numbering used is that of Pople, Schneider, and Bernstein.[17]

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[17] Pople, Schneider, and Bernstein, " High-resolution Nuclear Magnetic Resonance," McGraw-Hill, New York, 1959, p. 139.

which the position of attack is ambiguous. The only evidence given for the formation of apo-3-norbixinal methyl ester from natural bixin was the visible-light absorption maxima of a chromatographic fraction.

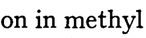
The analysis of the spectra of the two model esters (VIII) and (IX) requires comment. In both esters the protons of the terminal *C*-methyl groups are coupled to the adjacent olefinic protons and the spectra therefore depart from the four-spin system, A_2B_2. However, in both cases, the high-field band (see Figure) is little affected by the small interactions involving the methyl groups, and can therefore be analysed as an A_2B_2 system in which the positions of the lines yield explicitly the chemical shift and the four coupling constants.* The computations involved in the analysis were carried out with the English Electric DEUCE Computor. A programme was contructed in which line positions corresponding to various assignments of the half spectra (run at 40 Mc./sec.) were read in as raw data. The parameters were then computed and used to evaluate the eigenvalues and eigenvectors from which the theoretical line positions and intensities were derived; these were subsequently punched. In this way, it was possible to check rapidly a large number of possible assignments. The theoretical spectra corresponding to the best assignments are shown in the Figures. The spectrum of the " all-*trans* "-ester (VIII) was also run at 56·4 Mc./sec. and agreed well with the theoretical spectrum computed for this frequency. In the spectrum at 56·4 the bands arising from pairs of lines 6,11 of the A and B half-spectra overlap at the mid-point of the spectrum, and the band from the pair 7,10 of the B half-spectrum is clearly observable.

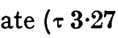
Chemical shifts (c./sec.) *and coupling constants* (c./sec.) *at* 40 Mc./sec. *for the olefinic protons in the triene esters* (VIII) *and* (IX).

		J_{34}	J_{35}	J_{36}	J_{45}
(VIII)	20·0	11·5	−0·6	±0·3	14·6
(IX)	47·1	12·5	−1·0	±0·2	10·7

The coupling constants and chemical shifts of the two triene esters are given in the annexed Table. The large coupling constants have been assumed to have positive values. The relative signs of J_{34} and J_{35} are significant. The spectra did not allow the relative signs of J_{36} and J_{45} to be decided, and in any case J_{36} is zero within experimental error. The values obtained for J_{45} in the two compounds prove the stereochemistry of the central double bonds to be that previously assigned from a consideration of the mode of preparation of the two isomers, and supported by their infrared absorptions. That the configuration of the two remaining double bonds is as shown in the formulæ can be justified as follows. The low-field bands in the spectra of the triene esters must be assigned to the protons at $C_{(3)}$ and $C_{(6)}$ because of the observable broadening caused by coupling with the methyl protons. If the terminal double bonds had the opposite configuration to that shown the protons at $C_{(4)}$ and $C_{(5)}$ would have been found at the lower field; studies on the three isomeric dimethyl muconates show that a methoxycarbonyl group deshields a *cis*-γ-proton more efficiently than a *cis*-β-proton.[19] Further, the two olefinic protons of the acetylenic precursor (X) of the triene esters absorb at 3·19 which is almost identical with the position of absorption of the olefinic proton in methyl tiglate (τ 3·27; cf. methyl angelate, τ 4·02).[1] The presence of the acetylenic group does not invalidate this comparison since the olefinic protons in 2-methylbut-2-ene (XI) [12] and 4-methylpent-3-en-1-yne (XII) absorb at 4·79 and 4·83 respectively.

(XI)





(XII)

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* The parameter $K = J_A + J_B$ [17] can be expressed explicitly in terms of line positions by taking advantage of the diagonal sum rule.[18]

[18] Condon and Shortley, " The Theory of Atomic Spectra," Cambridge University Press, 1953, p. 19.

[19] Jackman and Elvidge, *Proc. Chem. Soc.*, 1959, 89.

EXPERIMENTAL

Nuclear Magnetic Resonance Spectroscopy.—All spectral measurements were made on *ca.* 5% solutions in deuterochloroform at 21° by using a Varian Associates spectrometer (40 and 56·4 Mc./sec.). Tetramethylsilane was employed as an internal reference and the spectra were calibrated by the side-band technique with a Muirhead decade oscillator. The precision of most measurements was ±0·025 p.p.m. The accuracy of measurements on the triene esters (Table) was ±0·3 c./sec.

Methyl 2,7-*Dimethylocta*-2,4,6-*trienoate.*—The *trans,cis,trans*- (IX) and "all-*trans*"-isomer (VIII) were prepared by the method of Mildner and Weedon.[16] The *cis*-isomer had $\nu_{max.}$ (in $CHCl_3$) 1701 (CO_2Me) and 1608 cm.$^{-1}$ (C=C stretch). The "all-*trans*"-isomer had $\nu_{max.}$ (in $CHCl_3$) 1696 (CO_2Me), 1620 (C=C stretch), and 974 cm.$^{-1}$ (*trans*–CH=CH–, out-of-plane C–H deformation).

cis-*Apo*-1-*norbixinal Methyl Ester* (IV).—Oxidation of natural bixin (207 mg.) by the method of Karrer and Solmssen,[6] chromatography of the crude product on alumina (Grade IV) from benzene, and crystallisation from methanol, gave the *cis*-apo-1-ester (15 mg.), m. p. 148—149° (evacuated capillary, uncorr.), $\lambda_{max.}$ (in C_6H_6) 456 and 485 mμ (10^{-3} ε 93·4 and 83·5 respectively), $\nu_{max.}$ 1697 and 1664 cm.$^{-1}$ (ε 410 and 940, conjugated CO_2Me and conjugated CHO, respectively), τ (determined at 40 Mc./sec.) 8·00 with an inflexion at 8·10 (*C*-Me), 6·20 (CO_2Me) and 0·50 (CHO), with the expected relative intensities (for bands due to olefinic protons, see text). Karrer and Solmssen [6] give m. p. 156°, $\lambda_{max.}$ (in light petroleum), 441 and 470 mμ.

Neomethylbixin C.—The method of Zechmeister and Escue [8] was modified in the following way. A solution of methyl natural bixin (200 mg.) in hot benzene (100 ml.) was boiled under reflux for 1 hr., evaporated (to 10 ml.) under reduced pressure, and cooled. The methyl natural bixin (110 mg.) which crystallised was collected and treated as above; the recovered methyl natural bixin (55 mg.) was again recycled. The combined mother-liquors were evaporated (to 5 ml.). Repeated chromatography on alumina (Grade IV) from benzene and finally collection of the lower yellow band, evaporation, and crystallisation from benzene–methanol gave neomethylbixin C (15 mg.), m. p. 155°, $\lambda_{max.}$ 440, 465, and 498 mμ, $\nu_{max.}$ 1691 cm.$^{-1}$ (ε = 880), τ (determined at 56·4 Mc./sec.) 8·02 (*C*-Me) and 6·23 (CO_2Me) with the expected relative intensities (for bands due to olefinic protons, see text). Zechmeister and Escue [8] give m. p. 150—151°.

4-*Methylpent*-3-*en*-1-*yne* (XII) (with P. E. SCHWED).—Phosphorus oxychloride (10 ml.) in pyridine (20 ml.) was added in 30 min. to a stirred solution of 2-methylpent-4-yn-2-ol [20] (8·0 g.) in pyridine (20 ml.) at 0°. The mixture was stirred at 20° for 30 min. and then poured on concentrated hydrochloric acid (30 ml.) and ice. The upper hydrocarbon layer was separated, washed with water, dried (Na_2SO_4), and distilled, giving the acetylene (2·0 g., 30%), b. p. 71—74°/760 mm., n_D^{21} 1·4336, $\lambda_{max.}$ (in EtOH) 225 mμ ($E^{1\%}_{1\,cm.}$ 780), which was shown by gas–liquid chromatography to contain small amounts of impurities. Conversion into the ***mercury derivative***, which crystallised from alcohol and had m. p. 162° (Found: C, 40·25; H, 4·2. $C_{12}H_{14}Hg$ requires C, 40·25; H, 3·9%), and regeneration with 30% aqueous cyanide at 60—70°, gave 4-methylpent-3-en-1-yne, b. p. 72—73°/760 mm., n_D^{21} 1·4340, $\lambda_{max.}$ (in EtOH) 225 mμ (ε 8600), $\nu_{max.}$ 3268 and 2105 cm.$^{-1}$ (C≡CH), τ (determined at 40 Mc./sec.) 8·26, 8·21 (*C*-Me), 7·32 (conjugated C≡CH), and 4·83 (olefinic proton). Mondon [21] gives b. p. 73—75°.

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SOUTH KENSINGTON, LONDON, S.W.7. [*Received, October* 18*th*, 1960.]

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[20] Henbest, Jones, and Walls, *J.*, 1949, 2696.
[21] Mondon, *Annalen*, 1952, **577**, 181.