B were carried out with 10 g. of amalgamated zinc in 10 ml. of ethanol, 7.5 ml. of water and 10 ml. of concentrated hydrochloric acid. Small additions of hydrochloric acid were made during the 23-hour refluxing period. The product in each case was worked up as described in the reduction of the keto acid VII. From mixture A, 66 mg. of the diamino lactone XVII was obtained, m.p. 64–67°. After further purification by chromatography on alumina and elution with 1:1 chloroform-benzene solution, 56 mg. (14%) of XVII was obtained having ultraviolet and infrared spectra identical to that of the XVII isolated before. From mixture B 43 mg. (11%) of XVII was recovered.

Catalytic Reduction of Mixture B.—Mixture B (0.30 g.) was reduced with 0.01 g. of platinum oxide in 100 ml. of ethanol at atmospheric pressure and room temperature until hydrogen uptake had ceased (6 hours). The alcoholic solution was filtered and evaporated *in vacuo* at 50° to a light brown resin. This was dissolved in a small volume of ether from which it soon began to crystallize. This was recrystallized 6 times from methanol. The product (126 mg., 42%) proved to be the nitro amine XIII, m.p. 287° dec.; mixed m.p. 287° dec.

NEW HAVEN, CONNECTICUT

[CONTRIBUTION NO. 1912 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

Infrared Spectra and *cis–trans* Configurations of Some Carotenoid Pigments

BY K. LUNDE AND L. ZECHMEISTER

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Infrared spectra in relationship to *cis–trans* configurations are reported for some representatives of the following stereoisomeric sets: β-carotene, zeaxanthin, lycopene, α-carotene, γ-carotene, dimethylcrocetin and methylbixin. The influence of the spatial configurations on these spectra is manifest in the regions ~7.25, 10.0–10.6 and ~13 μ. Considering the new data, some configurations proposed earlier on the basis of spectral phenomena, observed in the visible and ultraviolet regions, have been confirmed while some others had to be revised.

The aim of the present paper is duofold. On one hand, we wish to contribute to the correlation of infrared spectra and *cis–trans* configurations of polyenes and, on the other, we endeavored to test, and where necessary to revise, in the field of the carotenoids, our earlier configuration assignments which were made without the benefit of the information given by infrared spectra.

While some reliable data have been reported concerning infrared spectra of *cis–trans* isomers of short conjugated systems,[1] relatively little is known about the influence of the configuration on vibrational spectra of long-chain polyenes.[2] Recently, we have studied along these lines the available members of the stereoisomeric diphenyl-ethylene, -butadiene, -hexatriene and -octatetraene sets,[2] after some new *cis* forms of the two latter compounds had been prepared in our laboratory.[3]

It has been pointed out[2] that the influence of the spatial configuration of a diphenylpolyene on its infrared curve becomes manifest in the following three regions: 7.0–7.1 μ (in-plane vibration of CH being part of a *cis* CC double bond); 12.84–12.95 μ (the analogous out-of-plane vibration); and 10.0–10.6 μ (out-of-plane vibration of CH in the corresponding *trans* grouping).

When evaluating our curves the following two features should be taken into consideration: (a) whereas in a diphenylpolyene molecule each C═C carries two hydrogens, in carotenoids several aliphatic double bonds carry a hydrogen and a methyl (*cf.* the formulas); (b) infrared spectra of diphenylpolyenes contain a great number of bands due to the presence of phenyl groups, while the carotenoid curves are simpler, although some configuration assignments encounter serious difficulties.

For the sake of convenience we will introduce the following terms: "methylated double bond" for a double bond located in a $—(CH_3)C═CH—$ group, and "unmethylated double bond" for one in a —CH═CH— group.

In the present paper we will discuss first the symmetrical C_{40}-carotenoids, β-carotene $C_{40}H_{56}$, zeaxanthin $(HO)C_{40}H_{54}(OH)$, and lycopene $C_{40}H_{56}$; then two non-symmetrical ones, *viz.*, α-carotene $C_{40}H_{56}$ and γ-carotene $C_{40}H_{56}$; and, finally, the lower-molecular symmetrical pigments, dimethylcrocetin $CH_3OOC{\cdot}C_{18}H_{22}{\cdot}COOCH_3$, and methylbixin $CH_3OOC{\cdot}C_{22}H_{26}{\cdot}COOCH_3$.

While Fig. 1 gives a general impression of an infrared carotenoid curve, in Figs. 2–4 only the stereochemically significant regions appear.

In the numbering of the carbon atoms we are following Karrer's nomenclature (*cf.* the β-carotene formula).

Stereoisomeric β-Carotenes (I) (Figs. 1–2).—Four spatial forms have been studied in this set, *viz.*, all-*trans*-β-carotene (natural product); central-mono-*cis*-β-carotene (absolute configuration established by Inhoffen, *et al.*, by total synthesis[4]); and the neo forms U and B obtained by iodine catalysis of the all-*trans* compound.[5,9]

Within the 10.0–10.6 μ region some bands (designated below briefly as "*trans*"-bands) have been assigned by earlier authors[6] to out-of-plane vibrations of the two hydrogen atoms contained in a *trans* C—CH═CH—C grouping. It is also known that conjugation of this grouping to an analogous *cis* group, *i.e.*, the formation of a *trans–cis*-diene

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(1) J. E. Jackson, R. F. Paschke, W. Tolberg, H. M. Boyd and D. H. Wheeler, *J. Am. Oil Chemists' Soc.*, **29**, 229 (1952); W. D. Celmer and I. A. Solomons, THIS JOURNAL, **75**, 3430 (1953); N. Sheppard and D. M. Simpson, *Quart. Revs.* (*London*), **6**, 1 (1952); R. S. Rasmussen and R. R. Brattain, *J. Chem. Phys.*, **15**, 131 (1947).

(2) K. Lunde and L. Zechmeister, *Acta Chem. Scand.* **8**, 1421 (1954); brief preliminary report, L. Zechmeister, *Experientia*, **10**, 1 (1954).

(3) K. Lunde and L. Zechmeister, THIS JOURNAL, **76**, 2308 (1954); L. Zechmeister and J. H. Pinckard, *ibid.*, **76**, 4144 (1954).

(4) H. H. Inhoffen, F. Bohlmann, K. Bartram, G. Rummert and H. Pommer, *Ann.*, **570**, 54 (1950).

(5) For the literature referring to the preparation and interpretation of *cis*-carotenoids *cf.* the survey article, L. Zechmeister, *Chem. Revs.*, **34**, 267 (1944).

(6) N. Sheppard and G. B. B. M. Sutherland, *Proc. Roy. Soc.* (*London*), **A196**, 195 (1949).

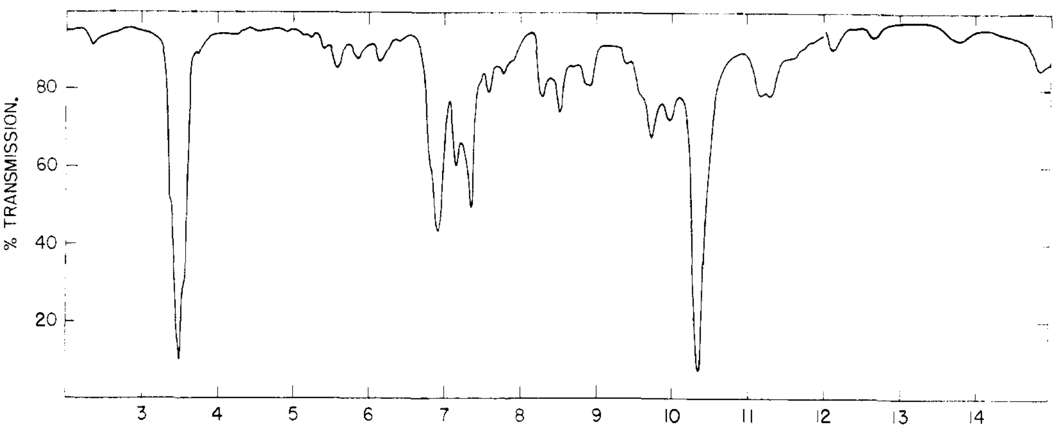
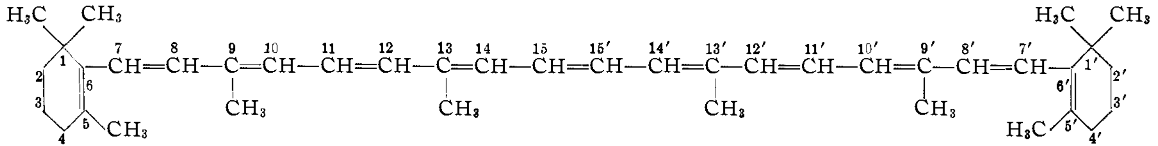
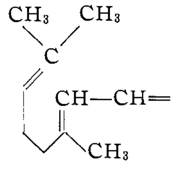


Fig. 1.—Infrared curve of all-*trans-β-carotene* from 2.0–15.0 μ (1 mm. cells): 1% carbon tetrachloride solution in the 2.0–12.0 μ region, and 0.5% cyclohexane solution in the 12.0–15.0 μ region.

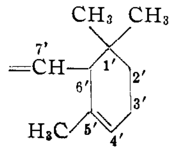
system, causes the split of the peak mentioned into a doublet[1] (in one instance a triplet has been reported[7]). We have observed such doublets in the spectra of some lower-molecular diphenylpolyenes but have predicted that in longer systems the doublet might be obscured.[2]



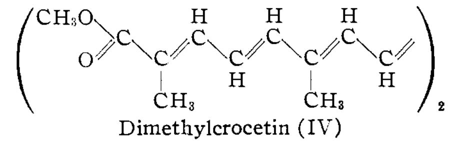
β-Carotene (I)



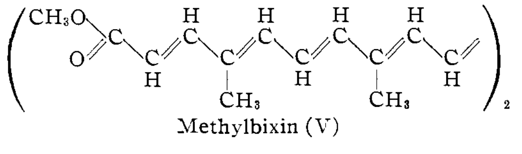
(II) Two end groups in lycopene; one such group in γ-carotene (the other end group is identical with those of β-carotene)



(III) One end group in α-carotene (the other end group is identical with those of β-carotene)



Dimethylcrocetin (IV)



Methylbixin (V)

So far as we know the group C—CH=CH—CH=CH—C is very rarely found in naturally occurring carotenoids; instead, the aliphatic section of the carotenoid molecule contains as a rule a number of units such as C—(CH$_3$)C=CH—CH=CH—C (*cf.* the β-carotene formula). Hence, it was not surprising that the neo-β-carotenes B and U showed only singlets, *viz.*, at 10.35 μ. When, however, in β-carotene the central double bond (adjacent to two —CH= groups) had assumed *cis* configuration, a doublet did appear, *viz.*, at 10.35 and 10.47 μ (Fig. 2).

The central-mono-*cis*-β-carotene curve contains the 10.35 μ "*trans*"-band which shows markedly less intensity than in the three other β-carotene curves (Fig. 2), in accordance with the presence of only four *trans* C—CH=CH—C groups in this isomer, *vs.* five in the all-*trans*, neo U and neo B molecules.

The neo-β-carotene U and B curves show a distinct band at 7.25 μ that is missing in the central-mono-*cis* and all-*trans* spectra. This is in accordance with some observations made in other stereoisomeric sets. Indeed, the 7.25 μ peak is missing in each all-*trans*-carotenoid curve studied so far, and, furthermore, in the spectrum of each such *cis* isomer whose *cis* double bond(s) is unmethylated (*e.g.*, central-mono-*cis*-β-carotene and central-mono-*cis*-methylcrocetin). Some special qualifications concerning this band will have to be made, however, for all-*trans*-β-carotene (see below). The 7.25 μ band is located within the region assigned earlier[8] to deformation vibrations of methyl groups.

The two double-bonded central carbon atoms in a carotenoid molecule do not carry methyls and thus no all-*trans* or central-mono-*cis* form can contain a methylated *cis* double bond. Since the 7.25 μ band is absent from the curves of such compounds, we propose that it be assigned to deformation vibrations of methyl groups attached to a *cis* double bond in an open-chain conjugated system.

Some other bands observed in the region 6.75–7.5 μ should be assigned, on the basis of Sheppard and Sutherland's studies,[6] to other deformation frequencies of methyl groups. The same considerations are valid for the carotenoids discussed below.

We note that the 7.0–7.1 μ band, assigned in diphenylpolyenes to in-plane vibrations of hydro-

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(7) W. D. Celmer and I. A. Solomons, This Journal, **75**, 3430 (1953).

(8) R. S. Rasmussen and R. R. Brattain, *J. Chem. Phys.*, **15**, 120 (1947).

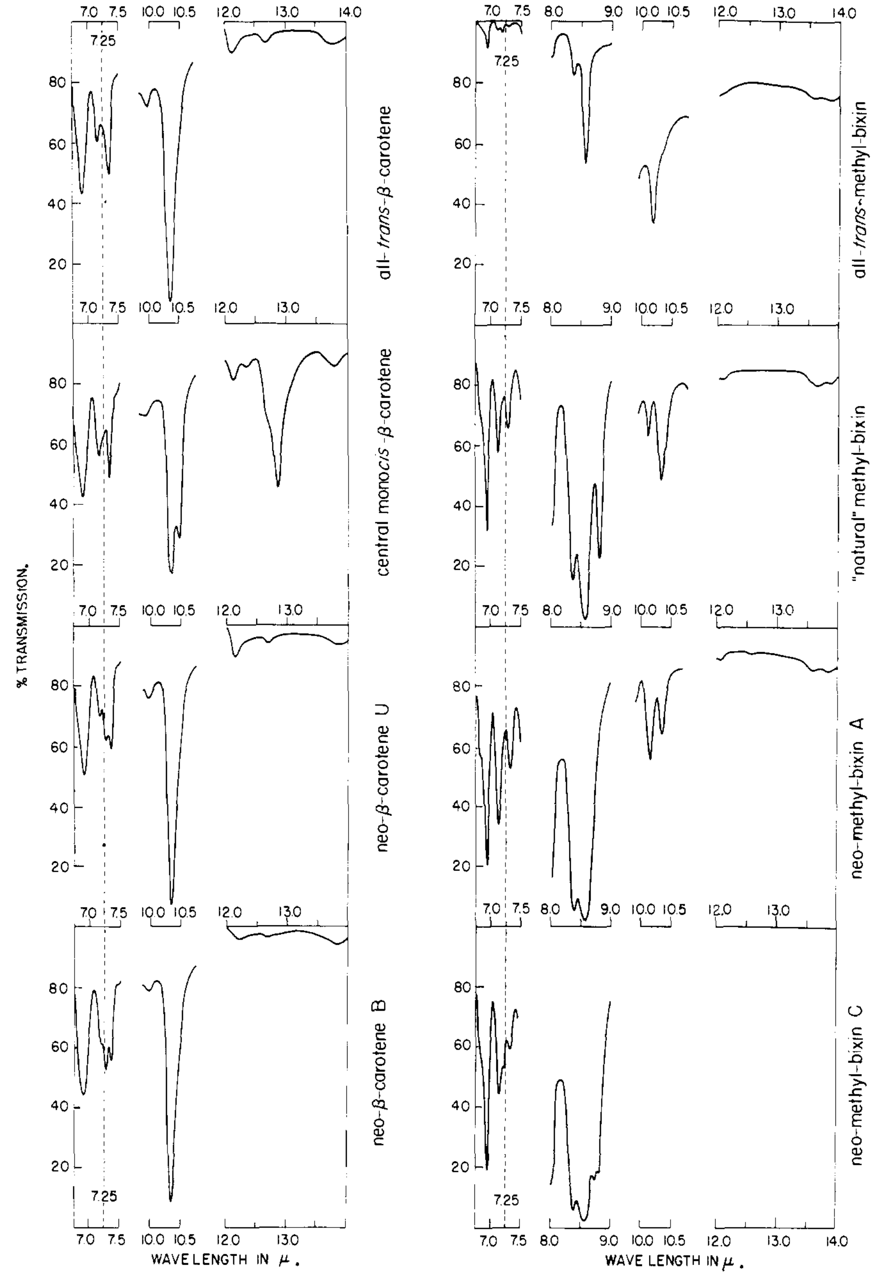

Fig. 2.—Stereochemically important sections taken from infrared curves of some *cis–trans* isomeric β-carotenes and methylbixins. β-Carotenes: 1.0% carbon tetrachloride solutions (1 mm. cell) in the 7.0–7.5 μ and 10.0–10.5 μ regions; and 0.5% cyclohexane solutions (1 mm. cell) in the 12.0–14.0 μ region, except for the central-mono-*cis*-isomer whose solubility in this solvent permitted the use of a 1.0% solution. Methylbixins: saturated carbon tetrachloride solutions (1 mm. cell) in the 7.0–7.5 μ and 8.0–9.0 μ regions; mineral oil mulls in the 10.0–10.5 μ and 12.0–14.0 μ regions (the corresponding sections of the neo C curve are missing because of lack of material).

gen atoms attached to *cis* double bonded carbon, is missing or is obscured in carotenoid spectra.

As mentioned briefly earlier,[2] central-mono-*cis*-β-carotene shows a strong band at 12.84 μ which is absent from the three other β-carotene curves given in Fig. 2. The interpretation of this band as a hydrogen out-of-plane vibration characteristic for a *cis* C—CH=CH—C group is in accordance with its presence in the curves of central-*cis*-carotenoids such as central-mono-*cis*-β-carotene and central-mono-*cis*-dimethylcrocetin or central-mono-*cis*-methylbixin (see below). It is not expected to appear in the spectra of those stereoisomers which either do not possess *cis* double bonds at all or contain methylated *cis* double bonds only.

In the light of this interpretation neo-β-carotene U must contain a methylated *cis* double bond and cannot contain a *cis* C—CH=CH—C group. Hence, *cis* configurations about the double bonds 7, 11, 15, 7′ and 11′ are excluded, in accordance with the 9-mono-*cis* configuration which was tentatively proposed for neo-β-carotene U on the basis

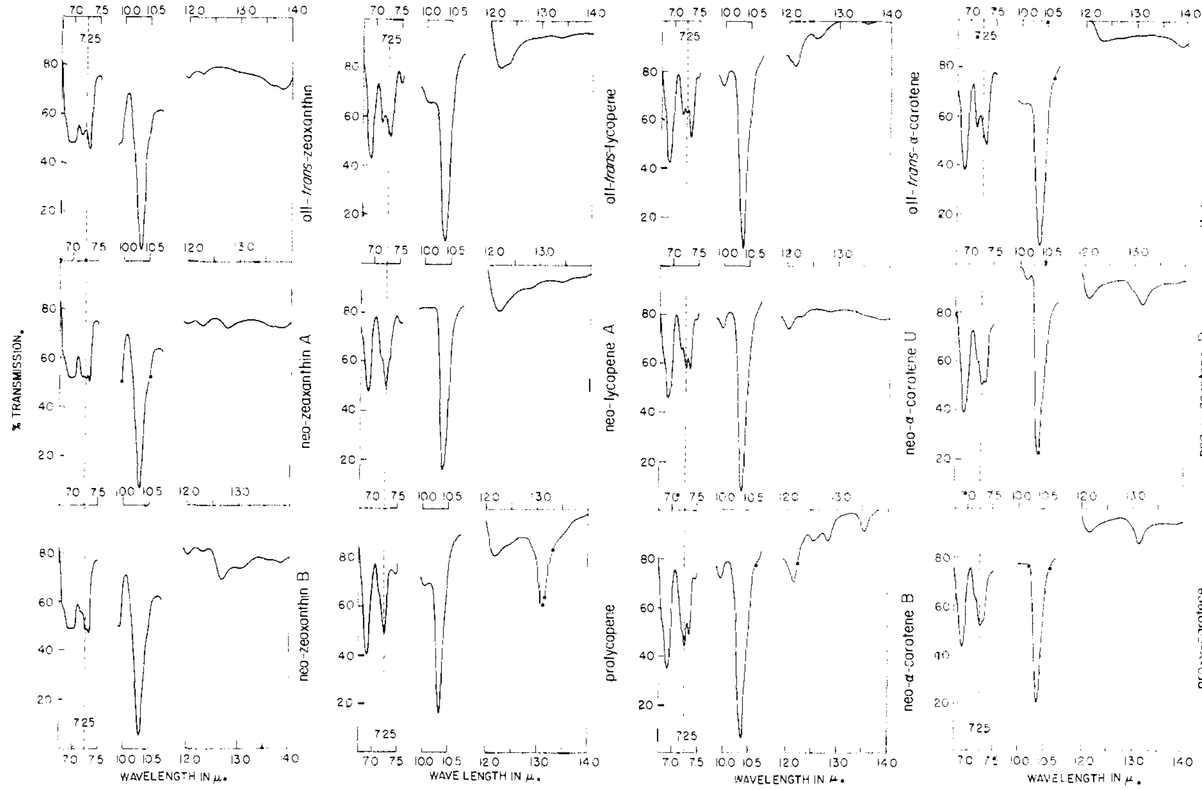

Fig. 3.—Stereochemically important sections taken from infrared curves of some *cis–trans* isomeric zeaxanthins, lycopenes, α-carotenes and γ-carotenes. Zeaxanthins: 1% chloroform solutions (1 mm. cell) in the 7.0–7.5 μ region; and mineral oil mulls in the 12.0–14.0 μ region. Lycopenes: 1% carbon tetrachloride solutions (1 mm. cell) in the 7.0–7.5 μ and 10.0–10.5 μ regions, and 0.25% solutions (2 mm. cell) in the 12.0–14.0 μ region. α-Carotenes (1 mm. cell): 1% carbon tetrachloride solutions of the all-*trans* and neo U isomers; and a 1.3% solution of neo B in the 7.0–7.5 μ and in the 10.0–10.5 μ regions; saturated cyclohexane solution of all-*trans* and a 1% cyclohexane solution of neo B in the 12.0–14.0 μ region; mineral oil mull of neo U in the same region. γ-Carotenes: 1% carbon tetrachloride solutions (1 mm. cell) in the 7.0–7.5 μ and 10.0–10.5 μ regions; 0.25% cyclohexane solution (2 mm. cell) of the all-*trans* form in the 12.0–14.0 μ region; and 0.5% cyclohexane solutions (1 mm. cell) of the two *cis* compounds in the same region.

of some spectral phenomena observed in the visible and ultraviolet regions.[9]

The neo-β-carotene B spectrum has two characteristics in common with that of the U isomer, *viz.*, (a) the presence of a 7.25 μ band (appearing with markedly higher intensity than in the neo U curve), and (b) the absence of a strong band in the 12.0–14.0 μ region. Hence, neo-β-carotene B may well represent a di-*cis* form as has been suggested before[5,9]; however, it cannot contain unmethylated *cis* double bonds. Thus, our earlier assumption of the presence of a central-*cis* double bond in this isomer must now be abandoned. Likewise, *cis* configurations at the double bonds 7, 7′, 11 and 11′ must be excluded for the same reason but also because they represent sterically hindered types which would be incompatible with the high extinction values and fine structure of the neo B curve in the visible region. Consequently, only the double bonds 9, 9′, 13 and 13′ remain available for a *cis* assignment, corresponding to the following four di-*cis* configurations: 9,9′, 9,13, 13,13′ and 9,13′.[10] Since only the 9,13′-di-*cis* model is compatible with the very high *cis* peak (in the ultraviolet region), we propose that neo-β-carotene B is 9,13′-di-*cis*-β-carotene.

Stereoisomeric Zeaxanthins (Fig. 3).—As reported before,[11] this 3,3′-dihydroxy-β-carotene yields two main, easily crystallizable *cis* isomers, termed neo A and B, whose infrared spectra as well as that of all-*trans*-zeaxanthin appear in Fig. 3. Both *cis* curves show (with very similar intensities) the 7.25 μ band that is missing in the all-*trans* spectrum. Although the neo A and B curves differ from that of the all-*trans* form also in the 12.0–14.0 μ region, these minor differences could not possibly prove the presence of an unmethylated *cis* double bond in the neo A and B molecules. This is confirmed by the very similar intensities of the "*trans*"-bands located at 10.35 μ in the three zeaxanthin curves mentioned.

On the basis of some observations made in the visible and ultraviolet regions, referring especially to the relatively very high *cis*-peak of neozeaxanthin A, the neo forms A and B had been interpreted, respectively, as 15- and 13-mono-*cis*-zeaxanthin.[11] While the mono-*cis* nature of these isomers is in accordance with their infrared spectra, the absence of unmethylated *cis* double bonds excludes the

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[9] A. Polgár and L. Zechmeister, This Journal, **64**, 1856 (1942).

[10] *Cf.* the models VIII, V, X and VII in ref. 5, p. 279.

[11] L. Zechmeister and P. Tuzson, *Ber.*, **72**, 1340 (1939); L. Zechmeister and R. M. Lemmon, This Journal, **66**, 317 (1944).

presence of *cis* configuration in the 15-position. Furthermore, the *cis*-peak of neo A (in the ultraviolet), although it is the highest such peak observed in the zeaxanthin set, is still much lower than the corresponding peak of 15-mono-*cis*-β-carotene (see above). We propose that probably neozeaxanthin A is 13-mono-*cis*-zeaxanthin, and that the only possible configuration assignment for neo B is that of 9-mono-*cis*-zeaxanthin, because of the symmetry of the molecule and the postulate that the *cis* double bond must belong to the methylated type.

Stereoisomeric Lycopenes (II) (Fig. 3).—Investigated were the all-*trans* form (natural product), neolycopene A (main *cis* isomer observed *in vitro*),[11,12] and prolycopene (a poly-*cis* natural product).[12,13] The 7.25 μ band was found to be absent from the all-*trans* curve but present in that of neo A and (perhaps with higher intensity) also in the prolycopene spectrum. We believe that neolycopene A must contain a methylated *cis* double bond; accordingly, our earlier interpretation of this isomer (based on the very high *cis* peak at 362 mμ, in hexane) now has to be revised. We assign to neolycopene A the probable configuration of a 13-mono-*cis*-lycopene, the one whose methylated *cis* double bond is almost centrally located in the molecule (*cf.* the high *cis* peak mentioned). It should be stressed at this point that neither central-mono-*cis*-lycopene nor the (likewise symmetrical) central-mono-*cis*-β-carotene[4] appears in stereochemical equilibria obtained *in vitro*, by iodine catalysis, for example.

The prolycopene spectrum shows a spectacular band at 13.15 μ that does not occur in the lycopene or neolycopene curves. If the position of the 12.8 μ band could be proven to be constant in the spectra of all carotenoids containing the central double bond in the *cis* configuration, then the observed absence of this band would exclude the configuration mentioned in the prolycopene molecule. Hence, the latter would have to contain at least one hindered (unmethylated) *cis* double bond.

Considering the presence of both methylated and unmethylated *cis* double bonds in the prolycopene molecule and the high intensities of the corresponding bands of both types, our earlier interpretation of this pigment as a poly-*cis* compound has been confirmed by the present study. This is also in accordance with a report on the partial stereoisomerization of prolycopene catalyzed by unusually small amounts of iodine.[14]

Prolycopene differs at least in two respects from Karrer's synthetic mono- and di-*cis*-lycopenes of the sterically hindered type,[15] *viz.*, (a) prolycopene is thermostable and crystallizes easily; (b) its spectral curve is flat in the ultraviolet *cis* peak region while those of the synthetic products do show some minor peaks.[16]

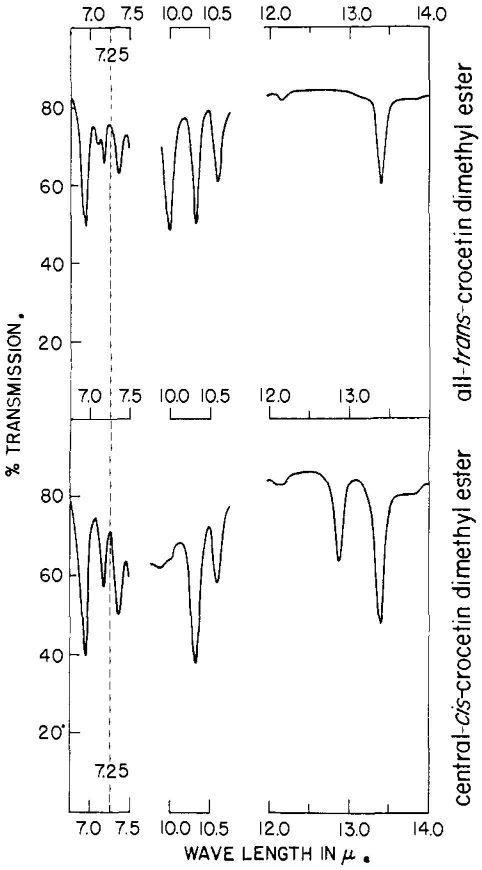

Fig. 4.—Stereochemically important sections taken from infrared curves of *cis–trans* isomeric dimethylcrocetins: saturated carbon tetrachloride solutions (1 mm. cell) in the 7.0–7.5 μ and 10.0–10.5 μ regions; mineral oil mulls in the 12.0–14.0 μ region.

Stereoisomeric α-Carotenes (III).—Figure 3 includes the all-*trans* form and two *cis* isomers, the neo-α-carotenes U and B.[17] In contradistinction to every other all-*trans*-carotenoid studied so far, the all-*trans*-α-carotene curve shows a slight but distinct peak at 7.25 μ.[18] This special feature can be explained as follows: The 7.25 μ peak is brought about by deformation vibrations of a methyl group attached to a *cis* double bonded carbon in an open chain. This situation is very similar to that of a methyl attached to the isolated double bond of a cyclohexene ring. We believe that the influence of such a double bond on the vibrations of a connected methyl group is similar to that of an aliphatic *cis* double bond. Thus, the appearance of the 7.25 μ band in all-*trans*-α-carotene had to be expected, in accordance with earlier observations made by Farrar, Hamlet, Henbest and Jones[19] who have pointed out the *cis* nature of a cyclic double bond in the infrared spectrum of vitamin A_2.

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[12] L. Zechmeister, A. L. LeRosen, W. A. Schroeder, A. Polgár and L. Pauling, THIS JOURNAL, **65**, 1940 (1943).

[13] A. L. LeRosen and L. Zechmeister, *ibid.*, **64**, 1075 (1942).

[14] Reference 13, p. 1078.

[15] C. F. Garbers and P. Karrer, *Helv. Chim. Acta*, **36**, 828 (1953).

[16] Besides prolycopene several other poly-*cis* compounds have been isolated from *Pyracantha* berries and none of them shows a *cis* peak; *cf.* L. Zechmeister and J. H. Pinckard, THIS JOURNAL, **69**, 1930 (1947).

[17] L. Zechmeister and A. Polgár, *ibid.*, **66**, 137 (1944).

[18] It should be noted in this connection that the inflection observed at 7.2 μ in the α-ionone spectrum is much more distinct than in the β-ionone spectrum. These observations were made using ionone samples purified *via* some derivatives, by K. Seitz, Hs. H. Günthard and O. Jeger, *Helv. Chim. Acta*, **33**, 2196 (1950). We have confirmed these data.

[19] K. R. Farrar, J. C. Hamlet, H. B. Henbest and E. R. H. Jones *J. Chem. Soc.*, 2657 (1952).

In Fig. 3 the intensity of the peak just mentioned increases in the sequence, all-*trans*-α-carotene < neo U < neo B, indicating the presence, respectively, of 0, 1 and 2 *aliphatic cis* $\mathrm{C{-}(CH_3)C{=}CH{-}C}$ groups.

The out-of-plane hydrogen vibration band usually located at 12.0–14.0 μ is missing in the neo-α-carotene U curve but the neo B spectrum does show some minor bands in that region. We believe that, possibly, certain minor stereoisomers present as contaminants may be held responsible for these bands, the more so since neo-α-carotene B crystallizates have not been obtained yet in stereochemically absolutely homogeneous state.

The absence of unmethylated *cis* double bonds in neo-α-carotenes U and B is confirmed by the practically identical intensity of the three respective "*trans*" bands at 10.35 μ observed in this set; it is, of course, also in accordance with the presence of the extensive fine structure in the visible region.

Evidently, our infrared data are in accordance with the earlier interpretation of neo-α-carotene U as the 9′-mono-*cis* form,[5,17] although the 9-mono-*cis* configuration cannot be excluded.

For neo-α-carotene B one of the two di-*cis* configurations, 13,9′ and 15,9′, was suggested earlier; however, the infrared data exclude the presence of an unmethylated *cis* double bond and hence the 15,9′ configuration. Thus, neo-α-carotene B is the 13,9′-di-*cis* or possibly (because of the asymmetry of the molecule) the 9,13′-di-*cis* isomer.

Stereoisomeric γ-Carotenes (II) (Fig. 3).—Three naturally occurring, crystalline isomers have been studied, *viz.*, all-*trans*-γ-carotene, pro-γ-carotene and neo-γ-carotene P.[16,20] Although pro-γ-carotene and neo P can be differentiated both chromatographically and by their visual spectra, surprisingly enough, their infrared spectra were found to be practically identical. A minimum of two *cis* double bonds must be present in either compound, since the curves contain the bands of both methylated and unmethylated *cis* double bonds. This is in accordance with the observation that the "*trans*" $\mathrm{C{-}CH{=}CH{-}C}$ band (10.35 μ) appears with considerably lower intensity in the pro-γ-carotene and neo P curves than in that of the all-*trans* form. The near-identity of the two *cis* curves indicated the same total number of *cis* double bonds. In this non-symmetrical stereochemical set there are several pairs of *cis* forms possible whose infrared spectra are expected to be (almost) identical; for example, two such compounds could contain the central double bond and, respectively, one of the adjacent double bonds in *cis* configuration.

We believe that our earlier interpretation of neo-γ-carotene P as a mono-*cis* form must now be revised. The new interpretation of the P-isomer as a di-*cis* compound is difficult to reconcile with the small wave length difference (3 mμ in hexane) from the all-*trans* form at ~460 mμ, considering the value of the corresponding difference (7 mμ) observed for neolycopene A. It is, however, possible that two adjacent *cis* double bonds located at the open end of the conjugated system in γ-carotene do have such small spectral effect. This situation must still be left open for discussion. (Possibly, the 3 mμ wave length difference mentioned would be understandable under the assumption that one of the methylated *cis* double bonds would be the terminal bond of the chromophore.)

There is no conclusive infrared spectroscopic evidence available at the present time of the poly-*cis* nature of pro-γ-carotene in contradistinction to that of prolycopene that clearly contains more than two *cis* double bonds.

Stereoisomeric Dimethylcrocetins (IV) (Fig. 4).—In this set the all-*trans* compound (isomerization product of methylated natural crocetin) and the synthetic central-mono-*cis*[21] isomers were investigated. As expected, both curves indicate the absence of a methylated *cis* double bond (at 7.25 μ). However, the two spectra are sharply differentiated by the presence of the strong 12.84 μ band in the curve of the *cis* compound, corresponding to an unmethylated *cis* double bond. Since these curves had to be taken from mulls of undetermined concentration, the observed "*trans*" bands cannot serve as a basis for comparative discussions. Neither are we able to offer an explanation for the apparently anomalous splitting of the "*trans*" band of the all-*trans* compound into a triplet.

Our experiments confirm the results of a recent remarkable study reported by Kuhn, Inhoffen, Staab and Otting[22] who observed the *cis* $\mathrm{C{-}CH{=}CH{-}C}$ band at 12.88 μ in the spectrum of central-mono-*cis*-dimethylcrocetin.

Stereoisomeric Methylbixins (V) (Fig. 2).—We have encountered considerable difficulties in the interpretation of the infrared spectra in this set whose following members were studied: the all-*trans* compound (obtained by isomerization of "natural" methylbixin); "natural" methylbixin (*cis*) prepared by methylation of naturally occurring bixin; and the neo A and C obtained *in vitro*. While the end groups of the dimethylcrocetin molecule are formulated as $\mathrm{CH_3OOC{-}C(CH_3){=}CH{-}}$, the corresponding two groups in methylbixin are $\mathrm{CH_3OOC{-}CH{=}CH{-}C}$.

We assume that in "natural" methylbixin one of the terminal C═C bonds is in *cis* configuration for the following two reasons: (a) Absence of a band at 7.25 μ and, hence, of a methylated *cis* double bond (this does not represent a strong argument in this series). (b) Presence of a strong band at 8.80 μ, in all probability representing a C–O stretching vibration; since it does not occur in the all-*trans* curve, the *cis* double bond of "natural" methylbixin must be held responsible for its appearance. We believe that the influence mentioned on the C–O stretching band must be caused by a double bond adjacent to the $\mathrm{CH_3OOC}$ group. This strongly indicates the terminal mono-*cis* configuration of "natural" methylbixin. Since this *cis* double bond is of the unmethylated type, *a priori* a band was expected to appear in the 12.0–14.0 μ region; its observed absence in this region could

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(20) J. H. Pinckard, Thesis, California Institute of Technology, 1949.

(21) H. H. Inhoffen, O. Isler, G. v. d. Bey, G. Raspé, P. Zeller and R. Ahrens, *Ann.*, **580**, 7 (1953).

(22) R. Kuhn, H. H. Inhoffen, H. A. Staab and W. Otting, *Ber.*, **86**, 965 (1953).

well be explained by the influence of the conjugated carbonyl group on the sensitive hydrogen out-of-plane vibration.

We believe that in this instance the sterically hindered *cis* double bond causes no loss in the fine structure of the ultraviolet spectral curve because of the particular character of the *cis* $CH_3OOC—CH=CH—$ group.

The very probable presence of a *cis* double bond adjacent to a CH_3OOC group in natural methylbixin was proposed also by Professor Linus Pauling who kindly communicated to one of us the following argument five years ago.

In general, when *cis* configuration is assumed about a sterically hindered double bond of a polyene, the steric hindrance would cause rotation around a neighboring single bond, so that the molecule would occupy two different planes and the conjugated system would split essentially into two such systems. However, if in "natural" methylbixin the double bond next to the CH_3OOC group were to have the *cis* configuration, there would be strong steric interaction between this group and the methyl group on the further carbon atom, and this would cause the carboxymethyl group to assume a configuration essentially normal to the plane of the rest of the molecule and would isolate the $C=O$ group from the conjugated system. Since CH_3OOC does not conjugate so strongly as a hydrocarbon double bond, the spectral difference observed in the visible region between the all-*trans* and the "natural" *cis* form (6 mμ) is not unreasonable.[23]

Although the *cis* nature of neomethylbixin A cannot be in doubt, considering especially its ultraviolet curve, and also indicated by a doublet at 10.15 and 10.35 μ, its spectral curve does not show a band expected either in the 7.25 μ or in the 12.0–14.0 μ region.[24] This feature cannot be explained at the present time, the less so since the neo C curve does show the 7.25 μ band. The latter curve also contains a band at 8.80 μ, *i.e.*, the same one that occurs in the "natural" methylbixin spectrum. We assume that one of the two *cis* double bonds contained in neo C molecules is identical with that of "natural" methylbixin—in confirmation of our earlier observation[25] that the main thermo- and photoisomerization product of "natural" methylbixin is neo C. The second *cis* double bond in the latter must represent the methylated type (band at 7.25 μ).

ADDENDUM.—Quite recently, we have investigated Inhoffen and Raspé's synthetic central-mono-*cis*-methylbixin.[24] As expected, the curve of this steric form does not contain the 7.25 μ band but shows a medium intensity band at 12.60 μ. The difference in the position of the latter from 12.84 μ (as observed in the spectra of the other central-mono-*cis* forms mentioned) could possibly be explained by the circumstance that, because of low solubility, a mull (instead of a solution) had to be used for this recording.

Acknowledgment.—We are indebted to Professor H. H. Inhoffen of the Technische Hochschule in Braunschweig, Germany, for pure crystalline samples of his synthetic central-mono-*cis*-β-carotene, central-mono-*cis*-methylbixin, and central mono-*cis*-dimethylcrocetin.

Experimental

The solvents used were Eastman Kodak Co. Spectro Grades. In each case fresh solutions were prepared and filled into the cells in dim light; exposure to shorter wave length light was avoided during recordings in the Perkin-Elmer self-recording infrared spectrophotometer, model 21. The mulls were obtained by preparing fine suspensions in Mineral Oil "No. 7"; they were sandwiched between sodium chloride crystals.

Neo-β-carotene B, neo-α-carotene B and neolycopene A that cannot be crystallized without at least some stereoisomerization, were rechromatographed on thoroughly prewashed columns. After transfer from the eluates into pure hexane, the (approximate) concentrations were determined in aliquot samples by means of iodine catalysis, in light, making use of the known extinction values at λ_{max} of such stereochemical equilibrium mixtures.

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[23] We may point out in this connection that, according to A. Smakula, *Angew. Chem.*, **47**, 657 (1934), in a polyene dicarboxylic acid only *one* –COOH shows the spectral effects of conjugation in the ultraviolet region. Thus, its "loss" (due to steric hindrance) would be hardly noticeable in that region.

[24] NOTE ADDED IN PROOF.—Recently, H. H. Inhoffen and G. Raspé (private communication) have succeeded in the total synthesis of central-mono-*cis*-methylbixin and have observed that its *cis*-peak (in the ultraviolet region) is much higher than that of our neomethylbixin A. Consequently, the latter cannot represent the central-mono-*cis* form.

[25] L. Zechmeister and R. B. Escue, THIS JOURNAL, **66**, 322 (1944).