[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 990]

Contributions to the Stereochemistry of γ-Carotene

BY L. ZECHMEISTER AND A. POLGÁR

The experimental conditions leading to the *cis–trans* isomerization of γ-carotene,[1] $C_{40}H_{56}$, as well as the characteristics of the resulting isomers, have been investigated but little. A naturally occurring stereoisomer, pro-γ-carotene, $C_{40}H_{56}$, has been isolated from several plants[2] and some of its steric alterations have been reported in collaboration with Schroeder but at the present time pro-γ-carotene cannot be obtained from the all-*trans* compound *in vitro*. A neo-γ-carotene zone with somewhat weaker adsorption affinity than that of all-*trans*-γ-carotene was observed by several authors.[3] Furthermore, it has been shown in collaboration with LeRosen, Schroeder and Pauling[4] that the extinction curve of γ-carotene undergoes the same typical alterations as other carotenoids when refluxed or catalyzed with iodine; the maxima in the visible region decrease and a "*cis*-peak"[5] appears at 349–350 mμ in hexane.

A sharp *cis*-peak is not observed in carbon disulfide solution but a general elevation of the extinction curve in this region does occur (Fig. 1).

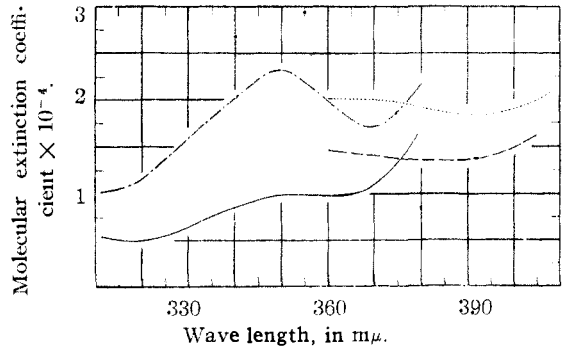


Fig. 1.—Molecular extinction curves of γ-carotene in the *cis*-peak region in hexane and carbon disulfide: ———, all-*trans*-γ-carotene in hexane; — · · —, iodine equilibrium curve in hexane; – – – –, all-*trans*-γ-carotene in carbon disulfide; . . . . . , iodine equilibrium curve in carbon disulfide.

This so far unexplained behavior is shown also by lycopene, $C_{40}H_{56}$, (Fig. 2) as well as by the bacterial pigment, spirilloxanthin,[6] $C_{40}H_{54}(OCH_3)_2$.

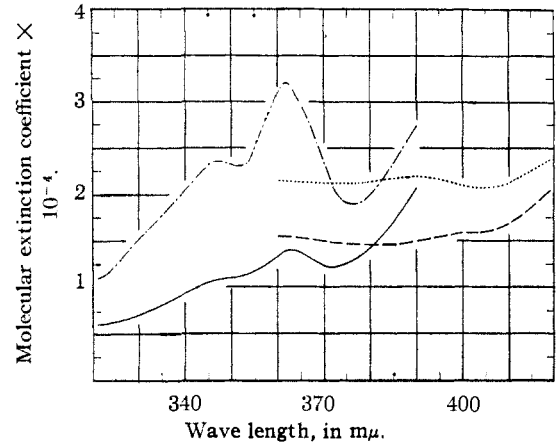


Fig. 2.—Molecular extinction curves of lycopene in the *cis*-peak region, in hexane and carbon disulfide: ———, all-*trans*-lycopene in hexane; — · · —, iodine equilibrium curve in hexane; – – – –, all-*trans*-lycopene in carbon disulfide; . . . . . , iodine equilibrium curve in carbon disulfide.

A more detailed discussion is given elsewhere.[7]

The refluxing of γ-carotene, like that of α- or β-carotene, yields chiefly isomers with decreased adsorption affinity. However, a minor stereoisomer, neo-γ-carotene U, is adsorbed above the all-*trans* form in the Tswett column, and corresponds to neo-α-carotene U and neo-β-carotene U.[8] The chromatographic separation of the isomers located below all-*trans*-γ-carotene is difficult because the interzones are narrow and, as a rule, not perfectly colorless. After a prolonged development, four zones appeared of which, however, the third and the fourth (located below neo A and B) were only partially resolvable mixtures (designated as Groups I and II). These difficulties are less than in the stereoisomeric gazaniaxanthin set.[9] The visual spectra of a number of isomers are practically identical in both sets.

The extent of stereoisomerization of the recovered pigment upon refluxing in petroleum ether under analogous conditions was: α-carotene, 8%; β-carotene, 14%; γ-carotene, 30%; and lycopene, 43%.

(1) R. Kuhn and H. Brockmann, *Ber.*, **66**, 407 (1933).

(2) (a) L. Zechmeister and W. A. Schroeder, THIS JOURNAL, **64**, 1173 (1942); *J. Biol. Chem.*, **144**, 315 (1942); (b) *Arch. Biochem.*, **1**, 231 (1943); W. A. Schroeder, THIS JOURNAL, **64**, 2510 (1942); L. Zechmeister and R. B. Escue, *J. Biol. Chem.*, **144**, 321 (1942).

(3) R. F. Hunter and A. D. Scott, *Biochem. J.*, **35**, 31 (1941); F. W. Went, A. L. LeRosen and L. Zechmeister, *Plant Physiol.*, **17**, 91 (1942); *cf.* footnote (2).

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

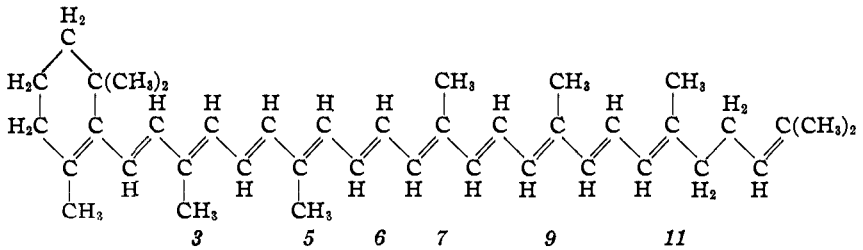
(5) L. Zechmeister and A. Polgár, *ibid.*, **65**, 1522 (1943).

(6) A. Polgár, C. B. van Niel and L. Zechmeister, *Arch. Biochem.* **5**, 243 (1944).

(7) L. Zechmeister, "*cis–trans* Isomerization and Stereochemistry of Carotenoids and Diphenylpolyenes," *Chem. Rev.*, **34**, 267 (1944).

(8) A. Polgár and L. Zechmeister, THIS JOURNAL, **64**, 1856 (1942); L. Zechmeister and A. Polgár, *ibid.*, **66**, 137 (1944).

(9) L. Zechmeister and W. A. Schroeder, *ibid.*, **65**, 1535 (1943).



All-*trans*-γ-carotene (the six stereochemically effective double bonds are numbered)

Iodine catalysis of γ-carotene at room temperature brings about a stereoisomeric mixture, the chromatogram of which has the characteristics described above. However, two main zones (neo A′ and B′) are not identical with the similarly located isomers (neo A and B) which were obtained by refluxing. They are again followed by two mixed zones (Groups I′ and II′). In contrast, α-carotene, β-carotene or lycopene produces only one main isomer with diminished adsorption affinity.

The extent of stereoisomerization of the pigment recovered after iodine catalysis was: α-carotene, 49%; β-carotene, 52%; γ-carotene, 44% and lycopene, 50%. The influence of illumination[10] is demonstrated in Fig. 3. A light impulse as short as five seconds caused a considerable stereochemical effect.

We emphasized[7] that in such studies "overexposure may cause destruction." This phenomenon which was recently confirmed in a detailed investigation by Zscheile, Harper and Nash[10a] does not prevent the use of iodine catalysis for the estimation of members of most stereochemical sets, under short exposure and well-established conditions as given in our papers. Indeed, the possible errors in the molecular extinction curves which are characteristic of the iodine equilibrium (or quasi-equilibrium) are essentially not greater than those in the chromatographic operations required for the analysis.

When exposed to sunshine[4] in petroleum ether for ninety minutes, the initial colorimetric value of γ-carotene decreased by 10% and the recovered pigment contained about 8% stereoisomers, *i. e.*, somewhat more than was found for β-carotene. The extent of stereoisomerization of lycopene was 28% in a parallel experiment. The total colorimetric loss (10%) showed that a formation of colorless cleavage products did not take place in any marked extent.

Some data concerning the steric behavior of γ-carotene in the melt and in acid catalyzed solutions are listed in the Experimental Part.

Our present incomplete knowledge of the configuration of some observed members of the stereoisomeric γ-carotene set can be summarized as follows.

The γ-carotene chromophore is stereochemically and structurally unsymmetrical.[4,7] The number of the double bonds which, according to Pauling[11] are able to assume *cis* configuration is six (see the formula), and the calculated number of stereoisomers is sixty-four. Eleven isomers are mentioned in the present paper, the two natural products, all-*trans*-γ-carotene and pro-γ-carotene included.

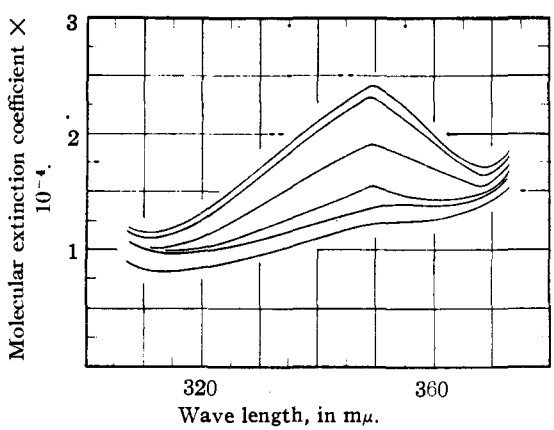


Fig. 3.—Influence of illumination on the development of the *cis*-peak effect in an iodine-catalyzed solution of γ-carotene, in hexane. Curves from bottom to top: solution before the addition of iodine, without illumination; upon iodine catalysis, after 0 sec., 5 sec., 30 sec., 2.5 min., and 15 min. illumination.

Pro-γ-carotene has been interpreted[4] as *3,5,7,-9,11*-penta-*cis*-γ-carotene with a single sterically effective (centrally located) double bond still in *trans* configuration. If pro-γ-carotene (visual maximum, 461 mμ) is kept in the melted state for one minute, a subsequent chromatogram shows two zones below the unchanged starting material. One of these (probably identical with a zone observed previously[2a]) has a maximum at 456 mμ and has been interpreted as *3,5,6,7,9,11*-hexa-*cis*-γ-carotene, briefly termed "all-*cis*-γ-carotene." The other zone (458.5 mμ) could contain one or more of the six possible penta-*cis* isomers. Like pro-γ-carotene, none of the two pigments mentioned shows a peak at 349–350 mμ, which is in accordance with our theoretical considerations.[4]

(10) A. Polgár and L. Zechmeister, THIS JOURNAL, **66**, 186 (1944).

(10a) F. P. Zscheile, R. H. Harper and H. A. Nash, *Arch. Biochem.*, **5**, 211 (1944).

(11) L. Pauling, *Fortschr. d. Chem. org. Naturstoffe*, **3**, 203 (1939); L. Zechmeister, A. L. LeRosen, F. W. Went and L. Pauling, *Proc. Natl. Acad. Sci.*, **27**, 468 (1941). For the numbering system *cf.* reference 7, pp. 272 and 276; on p. 318 (third line from bottom) the figures should read *3, 5, 6, 7, 9* and *11*.

It was pointed out earlier[4,8] that a single *trans*→*cis* rotation visually decreases the wave length of the longest wave length maximum of an all-*trans* carotenoid by about 5 mμ. Therefore, neo-γ-carotene U (Table I) must be a mono-*cis* compound. Its low *cis*-peak indicates that the *cis* double bond is peripherally located, either in *3* or in *9* position (*11* is less probable). The minor isomer, neo H, is evidently also a mono-*cis* compound; however, it contains a centrally located *cis* rotation visually decreases the wave length of peak the most probable configuration is that of *6*-mono-*cis*-γ-carotene although the *5* and *7* positions cannot be excluded.

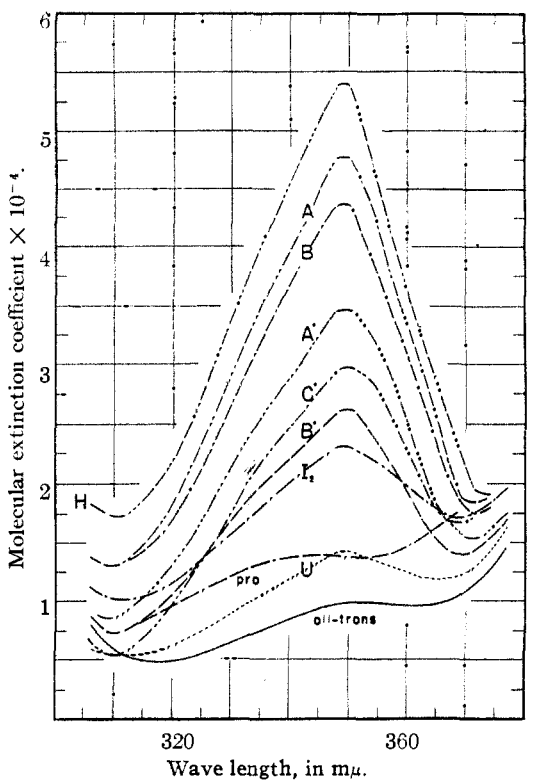


Fig. 4.—Molecular extinction curves of some γ-carotene stereoisomers in the *cis*-peak region, in hexane. (I_2 indicates the equilibrium mixture obtained upon iodine catalysis in light.)

The neo-γ-carotenes A, B, A′ and B′ (Table I) have practically identical visual spectra but they can be reliably differentiated by their unequal *cis*-peaks. We believe that they all are di-*cis* compounds whose configurations cannot be indicated at present with any degree of probability. Neo-γ-carotene G′ contains three (or perhaps only two) *cis* double bonds.

In addition to the experiments discussed we also report on the mild iodine catalysis of pro-γ-carotene,[12] and, furthermore, on the influence of carbon dioxide on the calcium hydroxide chromatogram of γ-carotene and some other carotenoids. This effect is not of a stereochemical nature.

(12) Similar experiments were reported for prolycopene, $C_{40}H_{56}$, by A. L. LeRosen and L. Zechmeister, THIS JOURNAL, **64**, 1075 (1942).

TABLE I

TYPICAL SPECTROSCOPIC DATA FOR SOME MEMBERS OF THE STEREOISOMERIC γ-CAROTENE SET

Member of the set[a]	Method by which obtained	Visually observed spectral maxima in petroleum ether (mμ)		Difference of the visually observed longest wave length maximum from that of the all-*trans* form in petroleum ether (mμ)	$E^{mol.}_{1\,cm.} \times 10^{-4}$ at the *cis*-peak wave length (349 mμ)	Difference between mol. ext. coeff. of the member and of all-*trans* form at the *cis*-peak wave length
Neo U	Iodine; refl.	489	457.5	5	1.4	0.4
All-*trans*	(Natural)	494	461.5	0	1.0	...
Neo A	Refluxing	486	455.5	8	4.8	3.8
Neo B	Refl.	486	455.5	8	4.4	3.4
Neo H	Refl.	489	457.5	5	5.4	4.4
Neo A′	Iodine	485.5	455	8.5	3.5	2.5
Neo B′	Iodine	486	455	8	2.6	1.6
Neo G′	Iodine	483	452	11	3.0	2.0
Pro-γ-carotene	(Natural)		461	33	1.4	0.4
All-*cis*	Melt		456	38	...	...
Penta-*cis*	Melt		458.5	35.5	...	...

[a] The first column does not give any chromatographic sequence. The homogeneity of the "Penta-*cis*" isomer has not been proved conclusively. A *cis*-peak estimation for the mixture of all stereoisomers adsorbed below neo B or neo B′ gave a remarkably high result, *viz.*, $E^{mol.}_{1\,cm.} = 3.8 \times 10^4$.

Experimental

(a) **Materials and Methods.**—In general the methods described earlier[7] were applied. For the development of chromatograms on calcium hydroxide (Shell Brand lime, Chemical hydrate; 98% through 325 mesh) petroleum ether (Skellysolve B, b. p. 60–70°) containing 2% acetone was used. However, about an hour is required for such a development on the scale of a 20 × 3.8 cm. column.

Our starting material, *viz.*, chromatographically homogeneous, crystalline γ-carotene was prepared by Dr. W. A. Schroeder from *Mimulus longiflorus* and partly from *Gazania rigens* flowers. The unexplained variations in the melting point of the samples were discussed earlier.[2b] The source of the crystalline pro-γ-carotene was the fruit of *Pyracantha angustifolia*. The molecular extinction coefficients of our samples in hexane were: γ-carotene, $E^{mol.}_{1\,cm.} = 14.4 \times 10^4$ at 462 mμ; and pro-γ-carotene, $E^{mol.}_{1\,cm.} = 10.0 \times 10^4$ at 457 mμ.

The use of the Beckman Photoelectric Spectrophotometer, the Zeiss Grating Spectroscope, and the Pulfrich Gradation Photometer was reported earlier.[5,8] The visually observed spectral data refer to petroleum ether solutions. Upon the addition of iodine all members of the γ-carotene set shifted their visually observed maxima to 490.5, 458 mμ (±0.5 mμ).

The determination of those isomers which were available in solution only was carried out after iodine catalysis and short illumination, on the basis of the values given for the iodine equilibrium extinction curve. The concentrations obtained were also used for the estimations of the individual *cis*-peaks.[5,8]

(b) ***cis–trans* Isomerization of γ-Carotene Solutions on Standing or Refluxing.**—A solution of 4 mg. of pigment in 25 ml. of petroleum ether was found to yield much less than 1% isomers when kept in diffuse daylight at 20° for two hours. After a day of standing the extent of the *trans* → *cis* isomerization, as in the cases of the two other carotenes, was 1 to 2%. Upon refluxing 3 mg. of γ-caro-

tene in 40 ml. of petroleum ether for thirty minutes in poor light, the following chromatogram was obtained (column, 20 × 3.8 cm.). (The figures on the left side denote width of zones, in mm.)

65 almost colorless
55 reddish-orange, unchanged all-*trans*: 494, 461.5 mμ
5 dark yellow (its top paler), neo A: 487, 456 mμ
2 slightly colored interzone
8 light orange, neo B: 486.5, 455 mμ
2 slightly colored interzone
15 light orange, mixture, Group I: 488, 457 mμ
17 light orange, mixture, Group II: 489, 457.5 mμ

The colorimetric ratios were, in two independent experiments, all-*trans* (including minor amounts of neo U):neo A:neo B:Group I:Group II = 73:6:5:8:8; and 70:14:4:8:4.

In contrast to chromatograms obtained after iodine catalysis (Section d), no clear separation of neo-γ-carotene U occurred when the top fourth of the all-*trans* layer was rechromatographed. Spectroscopic measurements nevertheless indicated the presence of very small quantities of this isomer which, upon repeated chromatography, depressed the wave lengths of the maxima by 3 to 4 mμ in the uppermost tenth of the all-*trans* zone. Group II yielded in a partial chromatographic resolution a homogeneous stereoisomer, neo H (489, 457.5 mμ), which is characterized by the pink color of its adsorbate.

(c) *cis–trans* **Isomerization of γ-Carotene by Melting Crystals.**—Three milligrams of pigment was kept fused in a sealed tube at 155° in a dibutyl phthalate-bath for fifteen minutes and then chromatographed (20 × 3.8 cm.):

27 almost colorless
5 light orange, neo U: 489, 458 mμ
2 slightly colored interzone
18 reddish-orange, unchanged all-*trans*: 492.5, 460 mμ
5 almost colorless
9 orange, no name: 486.5, 455 mμ
15 orange, mixture: 485.5, 453.5 mμ
37 yellowish orange, mixture: 484.5, 452.5 mμ

The ratio in the recovered pigment was, neo U:unchanged all-*trans*:stereoisomers adsorbed below the former = 4:39:57. In a second experiment 51% of the recovered pigment consisted of unchanged all-*trans*-γ-carotene (+ neo U).

(d) *cis–trans* **Isomerization of γ-Carotene by Iodine Catalysis at Room Temperature.**—A solution of 3 mg. of pigment in 25 ml. of petroleum ether was catalyzed with 0.06 mg. of iodine. After illumination with two 3500° white Mazda lamps (40 watt, length of the tubes 120 cm.) from a distance of 60 cm., for 10 to 15 min.; the following chromatogram was obtained (20 × 3.8 cm.).

45 colorless
45 reddish-orange, mainly unchanged all-*trans*: 493.5, 461 mμ
2 slightly colored interzone
10 light orange, neo A′: 485.5, 455 mμ
2 slightly colored interzone
12 orange, neo B′: 486, 455 mμ
18 top yellow, bottom orange, mixture, Group I′: 486.5, 455.5 mμ
10 light orange, mixture, Group II′: 485.5, 454.5 mμ

The uppermost third of the 45 mm. pigment zone when rechromatographed on a smaller column yielded upon prolonged development a top section with distinctly lighter orange color than the main, all-*trans* zone. The amount of this neo-γ-carotene U fraction was 3% (±1%) of the total recovered pigment and showed maxima at 489, 457.5 mμ.

The colorimetric ratio of the stereoisomers was then, neo U:unchanged all-*trans*:neo A′:neo B′:Group I′:Group II′ ≐ 3:53:7:14:16:7. In another experiment the ratio was, unchanged all-*trans* (including some neo U):neo A′:neo B′:Group I′:Group II′ = 58:7:11:16:8.

The zone of neo B′ proved to be homogeneous while a small amount of the all-*trans* pigment (perhaps formed by re-isomerization) separated out in a subsequent chromatogram from the neo A′ zone which then showed maxima at 486, 455 mμ. A rechromatography of Group II′ yielded among others a minor pigment, neo G′ (483, 452 mμ), with a purely yellow color of its adsorbate.

(e) *cis–trans* **Isomerization of γ-Carotene by Hydrochloric Acid Catalysis.**—A mechanical shaking of 3 mg. of pigment dissolved in 50 ml. of petroleum ether with 25 ml. of 12% hydrochloric acid for thirty minutes yielded a pigment mixture which, after washing and drying, gave a chromatogram (20 × 3.8 cm.) similar to that described in Section (d). The extent of stereoisomerization was about 40%. When commercial con. hydrochloric acid was used, the recovered pigment was composed as follows; unchanged all-*trans*:stereoisomers:irreversibly formed pigment = 47:38:15. The latter polyene was adsorbed near the bottom of the chromatogram and showed maxima at 475.5, 445.5 mμ; upon the addition of iodine its wave lengths decreased by 1 mμ. This pigment forms a well-defined yellow zone on the Tswett column when pure petroleum ether is used as a developer while in the presence of 2% acetone it passes into the filtrate. It showed maxima in the Beckman spectrophotometer at 474, 443 mμ. These values are near the earlier reported maxima for 5,6-dihydro-β-carotene,[13] *viz.* 474.5, 445 mμ. A sharp minimum appeared at 462 mμ in both cases.

(f) **Photochemical** *cis–trans* **Isomerization of γ-Carotene.**—The solution of 3.5 mg. of γ-carotene in 40 ml. of petroleum ether was exposed to sunshine in a transparent quartz tube at 26° for thirty minutes while a parallel sample was kept in darkness. The chromatographically established extent of isomerization was 3.5% in the recovered pigment of the insolated sample and 1.5% in the blank. In two other experiments the insolation was extended to ninety minutes with the result that 7 and 9.5% of the recovered pigment consisted of isomerized γ-carotene (in the blank, 0.5%).

(g) *cis–trans* **Isomerization of Pro-γ-carotene by Melting Crystals.**—A similar experiment was described earlier in collaboration with Schroeder.[2a] The previous time of melting (twenty minutes) was now shortened to one minute. In an experiment at 130° with 2.6 mg. of pigment the following chromatogram appeared (20 × 3.8 cm.).

7 colorless
2 pink, all-*trans*: 492.5, 459.5 mμ
30 very pale orange, neo-forms: 488.5, 456.5 mμ
35 orange, unchanged pro-γ-carotene: 460.5, (430) mμ
12 slightly colored interzone
20 yellow, all-*cis*: 456, (426) mμ
1 almost colorless
5 light orange, penta-*cis* member(s): 458.5 mμ

The total colorimetric loss amounted to about one-third of the initial value. The ratio in the recovered pigment was, all-*trans*:neo-forms adsorbed directly below the former:unchanged pro-γ-carotene:all-*cis*:penta-*cis* bottom zone = 9:8:60:19:4.

(h) *cis–trans* **Isomerization of Pro-γ-carotene by Iodine Catalysis at Room Temperature.**—An experiment in which a considerable amount of iodine (2.5% of the pigment) was used was qualitatively described in collaboration with

TABLE II

INFLUENCE OF THE PRO-γ-CAROTENE/IODINE RATIO ON THE RATIO OF THE STEREOISOMERS IN THE RECOVERED PIGMENT UPON TWO MINUTES OF ILLUMINATION AT 25°

(1 mg. of pro-γ-carotene in 20 ml. of petroleum ether in each case.)

Amount of iodine (% of pigment)	Colorimetric ratio, all-*trans*:neo-forms (adsorbed between all-*trans* and pro):unchanged pro-γ-carotene
0.001	5:~0:95
.01	12:11:77
.02	22:18:60
.1	36:23:41

(13) A. Polgár and L. Zechmeister, THIS JOURNAL, 66, 1528 (1943).

Schroeder.[2a] In the present paper we studied the influence of the quantity of the catalyst on the steric composition of the recovered pigment (Table II).

(i) **Influence of Carbon Dioxide on Some Calcium Hydroxide Chromatograms of Carotenoids.**—If a stream of carbon dioxide is passed through a petroleum ether solution of γ-carotene for five to thirty minutes, no noticeable stereoisomerization takes place. When the solution is poured on a column and developed with the solvent containing 2% acetone, a single zone moves downward with unusual speed at first, due to the formation of carbonate in the top section. Later, when this movement slows down because of the local absence of carbonate, a separation into two very well differentiated zones occurs. The lower zone is unchanged γ-carotene and the upper one is a "complex" which contains 15 to 30% of the initial pigment. The two adsorbates have very similar colors. The upper zone does not migrate further, even if the column is washed with pure acetone; furthermore, it cannot be eluted with alcohol, acetone, dioxane, pyridine, etc. We were able to elute only very small fractions which showed the spectral bands of γ-carotene. In contrast, the unchanged γ-carotene zone can be eluted easily, and gives rise to the formation of a new portion of the complex upon a repeated treatment with carbon dioxide.

These phenomena are not observed if before chromatography the carbon dioxide is removed from the γ-carotene solution by means of a nitrogen stream or evaporation *in vacuo*. They can be reproduced by keeping the pigment solution in a carbon dioxide atmosphere, originating either from a Kipp generator or from Dry Ice.

So far we have observed the appearance of the non-elutable pigment on calcium hydroxide columns only (or on a mixture of the hydroxide and calcium carbonate) but not on pure calcium carbonate, aluminum oxide, zinc carbonate, magnesium oxide, magnesium hydroxide or barium hydroxide. Among the carotenoids tested, lycopene, which requires some reinvestigation, behaves like γ-carotene, as does also the bacterial pigment spirilloxanthin.[6] β-Carotene showed a much smaller effect.

If calcium hydroxide is to be used in quantitative experiments, the solutions should not be kept under carbon dioxide but, preferably, under nitrogen in order to exclude autoxidation.

Summary

The *cis–trans* isomerization of γ-carotene, $C_{44}H_{56}$, (from *Mimulus* and *Gazania* flowers, lower melting form) has been studied by several methods. Some stereoisomers have been tentatively assigned configurations.

PASADENA, CALIFORNIA RECEIVED SEPTEMBER 18, 1944

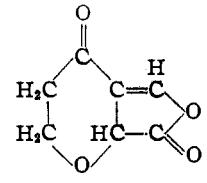
[CONTRIBUTION FROM THE DEPARTMENT OF MICROBIOLOGY, NEW JERSEY AGRICULTURAL EXPERIMENT STATION]

The Mechanism of the Antibiotic Action of Clavacin and Penicillic Acid[1,2]

BY WALTON B. GEIGER AND JEAN E. CONN[2a]

Despite the rapidly accumulating information concerning the production of antibiotic substances by microörganisms, their isolation, and their utilization for combating disease, comparatively little still is known of the mode of action of these substances upon bacteria. Among the most important characteristics of these substances is their selective action upon bacteria: some act largely upon Gram-positive forms and to only a very limited extent upon Gram-negative types, whereas others affect alike bacteria within both these groups. Among the substances that belong to the second category, clavacin[3,4,5,6] and penicillic acid[7] occupy a prominent place. Each of these substances is produced by several fungi. Both are active on bacteria belonging to the Gram-positive and Gram-negative types. Both clavacin (I) and penicillic acid (II) are α,β-unsaturated ketones.

Because of the comparatively simple structure of these two compounds, their peculiar antibacterial properties have aroused considerable attention. Of penicillic acid, Oxford[7] wrote," It is not too much to say that there is no feature, or combination of features, in this structure which, on the basis of existing knowledge, would lead one to anticipate an activity of the order found."



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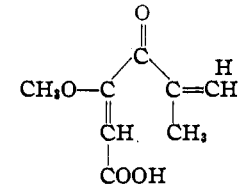

II

In the course of chemical studies on the structure of clavacin,[8] our attention was directed to a structural feature, common to both penicillic acid and clavacin, that seemed likely to be responsible for their antibacterial activity, namely, the —CH=C—C=O group. Moreover, this part of the molecule is the only structural detail common to both substances. The observation that drew attention to this grouping was the fact that clavacin is inactivated by sulfhydryl compounds such as cysteine or thioglycolate.

The ability of many α,β-unsaturated ketones to react with sulfhydryl compounds was discovered by Posner[9] and may be presented as follows

(1) Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University, Department of Microbiology.

(2) These investigations were supported by a grant supplied by The Commonwealth Fund of New York.

(2a) Present address, Department of Bacteriology, University of Iowa, Ames, Iowa.

(3) Waksman, Horning and Spencer, *J. Bact.*, **45**, 233 (1942).

(4) Raistrick, Birkinshaw, Michael, Bracken, Gye and Hopkins, *Lancet*, **245**, 625 (1943).

(5) Hooper, Anderson, Skell and Carter, *Science*, **99**, 16 (1944).

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