[CONTRIBUTION NO. 1588 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

Stereoisomeric Phytofluenes

BY F. J. PETRACEK AND L. ZECHMEISTER

Phytofluene *ex* tomatoes is subject to *cis–trans* isomerization. The native form in the tomatoes studied is a relatively thermostable but photosensitive *cis* compound that may rearrange spontaneously or when illuminated or under the catalytic influence of iodine in light. It then yields the more stable all-*trans* form possessing stronger adsorbability. The two main stereoisomers mentioned were characterized by spectroscopic and chromatographic methods.

The colorless polyene hydrocarbon, phytofluene, which fluoresces intensely in ultraviolet light and is a probable intermediate in the biosynthesis of carotenoids, has been investigated repeatedly.[1,2] Five years ago we made the brief statement that "it shows a marked tendency for *cis–trans* isomerization." In the present paper we wish to substantiate this claim and report some observations on the stereochemical properties of the tomato phytofluene studied.

When a small portion of the fresh, ripe fruit is extracted as rapidly as possible and chromatographed without delay, all operations being carried out in near darkness, then only a single main, greenish-gray fluorescing zone appears. However, in the course of lengthy operations or when the solution is kept standing, refluxed, or exposed to light, especially in the presence of iodine, a conversion takes place which yields, on adsorption analysis, a second (main) phytofluene zone. This reversible rearrangement leads in the presence of iodine to a stereoisomeric *quasi*-equilibrium mixture which contains (besides minor constituents) approximately 10% original phytofluene and 90% new phytofluene. The latter shows a markedly more intense fluorescence in ultraviolet light and much stronger adsorbability than were observed before the rearrangement.

The two compounds can be easily separated by a broad, non-fluorescing interzone when the chromatogram is developed on alumina with a 3:2 hexane–benzene mixture. Then the respective migration rates are so different that either of the two phytofluenes may be recognized on this basis alone, even in the absence of the other isomer. When α-carotene is also present, the top-to-bottom sequence is: α-carotene, interzone, new phytofluene, interzone, and native phytofluene. Instead of alumina, a mixture of alumina–calcium hydroxide can be used with advantage. On pure calcium hydroxide, with hexane containing 1.5% acetone as the developer, the two phytofluenes do not separate as well as on alumina, however, a narrow interzone mostly obtains; on this adsorbent α-carotene forms a mixed zone with the top phytofluene. In the system magnesia–hexane +3% acetone, we were unable to resolve a mixture of the two phytofluenes since they were not completely separated, although both appeared distinctly below α-carotene.

The phytofluene present in our tomato samples and showing weaker adsorbability is a *cis* compound for which we propose to retain the accepted term "phytofluene." According to all evidence available and considering observations made earlier on carotenoid pigments,[3] we believe that the stereoisomer possessing higher adsorption affinity is all-*trans* phytofluene and should be designated as such.

The phytofluene preparations (*ex* tomatoes) which have been investigated up to the present time were very likely mixtures of the two forms in which the all-*trans* configuration prevailed. This is explained, in part, by the photosensitivity of freshly extracted or eluted phytofluene which becomes especially manifest in dilute solutions, containing a few milligrams of substance per liter, for example. In darkness both forms show considerable stability even under the conditions of refluxing. The behavior of our freshly prepared and chromatographically tested, homogeneous tomato phytofluene (*cis*) is summarized in Table I.

TABLE I

STEREOISOMERIZATION OF TOMATO PHYTOFLUENE (*cis*) IN HEXANE SOLUTION CONTAINING 10–15 MG. OF SUBSTANCE PER LITER

Treatment (in Pyrex volumetric flasks)	Duration	Ratio, *cis*:*trans*, in the recovered mixture[a]
Standing in darkness at 26°	24 hrs.	45:1
Refluxing in darkness	30 min.	25:1
Exposures to direct sunlight of different intensities	15 sec.	1:50 to 1:3[b]
Exposure to rather intense, scattered daylight (window sill, 26°)	30 min.	1:16
Exposure to daylight lamp (for conditions, *cf.* Exper. part)	10 min.	3:1
Exposure to daylight lamp in the catalytic presence of iodine (for conditions, *cf.* Exper. part)	10 min.	1:9

[a] The data listed were obtained by developing 25 ml. of the treated solution on an alumina–calcium hydroxide–celite column with hexane–benzene 3:2 and subsequent spectrophotometric identification and estimation of the two separated and eluted zones. [b] A parallel experiment with a solution having five times higher concentration yielded the approximate ratio, 1:1.

When all-*trans* phytofluene was submitted to the thermic or photochemical treatments listed in the table, it showed significantly greater stability than the *cis* form. Thus, the following operations did

(1) L. Zechmeister and A. Polgár, *Science*, **100**, 317 (1944); L. Zechmeister and A. Sandoval, *Arch. Biochem.*, **8**, 425 (1945); THIS JOURNAL, **68**, 197 (1946); J. Bonner, A. Sandoval, Y. W. Tang and L. Zechmeister, *Arch. Biochem.*, **10**, 113 (1946); L. Zechmeister and F. Haxo, *ibid.*, **11**, 539 (1946).

(2) J. W. Porter and F. P. Zscheile, *ibid.*, **10**, 547 (1946); J. W. Porter and R. E. Lincoln, *ibid.*, **27**, 390 (1950). Porter and Zscheile wrote: "The colorless polyenes described in this paper are not the only ones occurring in tomatoes, for other fluorescent bands have been observed on MgO-Super-Cel columns. The latter are present in very much smaller quantities, and may be largely isomers of the fluorescent, colorless polyene."

(3) L. Zechmeister, *Chem. Revs.*, **34**, 267 (1944).

not lead, on subsequent chromatography, to the appearance of the *cis* zone: refluxing in darkness for 30 min., exposure to intense sunlight for 2 min. or to the daylight lamp for 60 min. In contrast, iodine catalysis in light resulted, within 15 min., in the formation of approximately the same stereoisomeric mixture as that obtained starting from the *cis* isomer (*cis*:*trans* = 1:9).

A significant factor which seems to have prevented earlier recognition of the true stereochemical status of tomato phytofluene is the circumstance that the spectral maxima in the main band of both described forms are located at almost identical wave lengths. While in carotenoid molecules a single *trans* → *cis* rotation (in hexane) would shift the position of the main maxima, for example, by 5 mμ toward the blue end of the spectrum, the corresponding shift in the phytofluene set was found to be as small as 1 mμ at the longest wave length maximum and about 0.5 mμ at $\lambda_{max.}$. On the other hand, in full analogy to the behavior of carotenoid pigments, the height of the main extinction maxima increases markedly when *cis*-phytofluene is catalyzed with iodine, in light, while the same treatment of the *trans* form causes the opposite effect (Figs. 1 and 2).

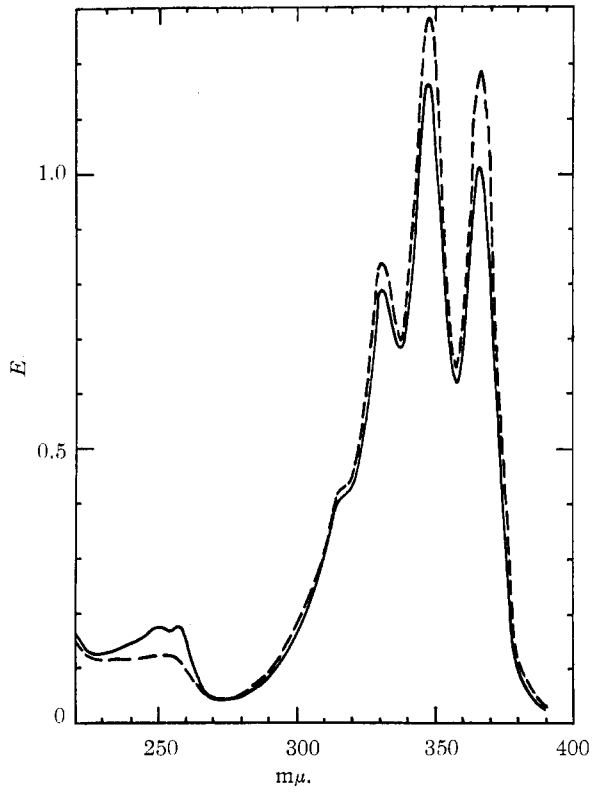

Fig. 1.—Extinction curve of tomato phytofluene (*cis*) in hexane: —, fresh solution; - - - -, after iodine catalysis, in light.

Furthermore, in accordance with some observations made on carotenoids, the spectral curve of all-*trans*-phytofluene possesses a higher degree of fine structure than that of phytofluene. This is illustrated by the almost equal heights of the two main maxima in the spectral curve of the *trans* compound.

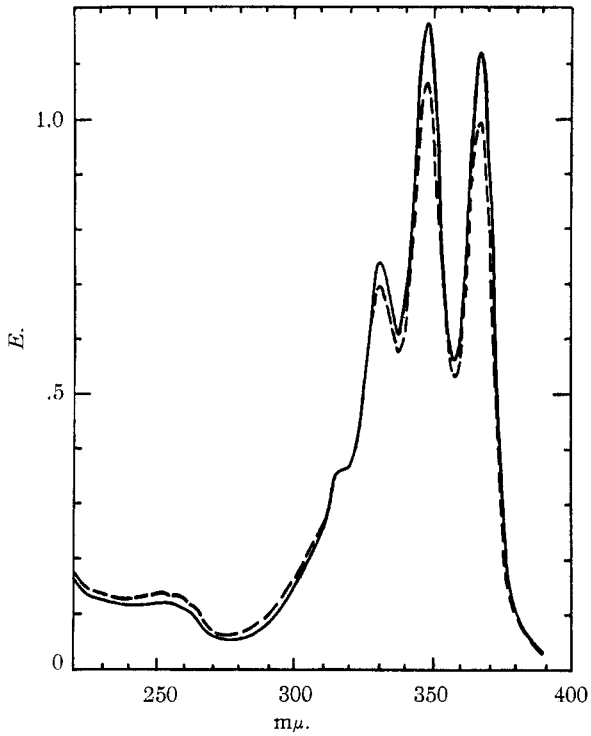

Fig. 2.—Extinction curve of all-*trans* phytofluene in hexane: —, fresh solution; - - - -, after iodine catalysis, in light.

As reported and interpreted earlier,[4] *trans* → *cis* bending of a carotenoid molecule causes the appearance of a "*cis*-peak" at about 142 mμ shorter wave length than that of the maximum which is nearest the red end of the spectrum. A similar observation has now been made in the phytofluene set, but the distance mentioned amounted here to only 115 mμ; this latter figure may well be compared with that (112 mμ) valid for another short conjugated system, *viz.*, 1,4-diphenylbutadiene.[5]

The *cis*-peak effect as demonstrated in Figs. 1 and 2 could be observed only with highly purified samples which had been submitted to repeated chromatography on lime. Otherwise, either impurities present in extracts or autoxidation products of the sensitive compound may cause disturbing extinctions in the region of 250–260 mμ where the (moderately high) *cis*-peak is located. Hence, the ratio, main maxima:*cis*-peak as taken from the curves, may still be subject to some revision.

On the basis of the reported data the most probable configuration of the tomato phytofluene studied is that of a mono-*cis* compound, in whose conjugated system the *cis* double bond does not seem to occupy central position.

We intend to study further the stereoisomeric phytofluene set, especially in order to clarify the status of minor, fluorescing zones whose eluates yielded phytofluene-like spectra. Furthermore, the amounts of the two main steric forms present in various plant materials will be tested.

(4) L. Zechmeister and A. Polgár, THIS JOURNAL, **65**, 1522 (1943); L. Zechmeister, A. L. LeRosen, W. A. Schroeder, A. Polgár and L. Pauling, *ibid.*, **65**, 1940 (1943).

(5) J. H. Pinckard, B. Wille and L. Zechmeister, *ibid.*, **70**, 1938 (1948).

The occurrence in plants of a *cis*-polyene as the preponderant or exclusive form is by no means an isolated phenomenon. In this connection we may mention bixin as well as the poly-*cis*-carotenoids, *e.g.*, prolycopene. Such configurations seem to be efficiently protected from stereoisomerization *in situ* which is illustrated anew by the photosensitivity of extracted phytofluene.

Experimental Part

Materials and Methods.—The tomato paste used was manufactured by the Campania West Coast Packing Company, Long Beach, Calif. The fresh tomatoes originated from markets and cannot be characterized genetically. As adsorbents calcium hydroxide "Sierra Hydrated Lime, Superfine" (U.S. Lime Products Corp., Los Angeles, Calif.) was used mixed 2:1 with Celite No. 545 (Johns-Manville Co.). The alumina was Alorco, Grade F, reground to −200 mesh and mixed in the proportion 4:1 with celite. For the differentiation of phytofluenes a mixture of alumina–calcium hydroxide–celite 3:1:1 can be recommended. The magnesia (Westvaco Chemical Div.) was mixed with celite 2:1. Solvents were freed of fluorescing contaminants by careful redistillation. Elutions were carried out with acetone in sintered glass funnels. All extractions, chromatographic resolutions, etc., should be conducted in very weak light. Photochemical treatments were carried out in Pyrex volumetric flasks. "Daylight lamp" means two 3500° white and yellowish Mazda lamps (40 watt, length, 120 cm.), applied from a distance of 60 cm. Spectral readings refer to hexane solutions in the Beckman spectrophotometer.

Extraction and Purification (A).- About 0.5 kg. of ripe, fresh tomatoes were ground in a Waring Blendor in the presence of 500 ml. of methanol, and the suspension was shaken mechanically for 30 min. The pulp was separated on a buchner funnel, with suction, and submitted to a single extraction by shaking on the machine with hexane–methanol 1:1 for 30 min. The methanol-free washed and dried (sodium sulfate) extract was immediately adsorbed on a 20 × 3.8-cm. alumina–celite column. Pigments and fluorescent substances were retained near the top but upon developing with hexane–benzene (3:2) rapid differentiation took place. The final sequence of the zones was lycopene and other pigments (top); interzone; a thin, strongly fluorescent streak (to be investigated) followed by and not well separated from, β-carotene; narrow interzone; all-*trans* phytofluene; a 5-cm. interzone; and phytofluene. Each of the two phytofluenes was, after elution, transferred into hexane and rechromatographed on a 20 × 3.8-cm. column. Both zones were then found to be chromatographically homogeneous. The relative concentrations were, *cis*:*trans* = 20:1. The *trans* form did not separate in the mixed-chromatogram test from a sample obtained from the *cis* compound by iodine catalysis and subsequent chromatographic purification.

(B).—Preparative amounts of the two phytofluenes were obtained as a by-product of a large scale lycopene isolation from commercial tomato paste as described earlier.[6] After the pigment had crystallized out, the combined mother liquors were concentrated *in vacuo*, adsorbed in large percolators on lime which was then washed with petroleum ether (b.p. 60–70°) containing 3% acetone, until the flow ceased to fluoresce in ultraviolet light. This chromatographic filtrate was concentrated *in vacuo* to a dark, viscous, oily residue which was dissolved in carbon tetrachloride and stored in glass tubes, sealed under nitrogen, in darkness in the cold room. By repeated chromatographic treatment of this concentrate on alumina as described most of the pigment was retained near the top of the column; below it two main fluorescing zones appeared, the ratio of which was 4:1 in favor of the bottom zone. Evidently, the *cis* configuration was still predominant in this sample.

This *cis* form could be purified easily as described under (A). More difficult was the purification of the *trans* isomer whose zone was contaminated by neo-β-carotenes. The latter could be removed by iodine catalysis (in hexane) and chromatographic elimination of the all-*trans*-β-carotene fraction formed. The all-*trans*-phytofluene thus obtained did not separate in the mixed-chromatogram test from a sample isolated either from the fruit according to Section (A) or prepared by iodine catalysis starting from the *cis* form. For analytical purposes the latter procedure is the preferred one.

Stereoisomerization by Means of Iodine Catalysis.—Twenty-five-milliliter samples of a hexane solution (*cis* or *trans*) containing 10–15 mg. of chromatographically homogeneous substance per liter, were catalyzed with iodine, whose amount corresponded to a few per cent. of the phytofluene, and exposed for 10 min. to the daylight lamp, under conditions stated above. Chromatography, elution and readings in the spectrophotometer gave the approximate ratio *cis*:*trans* = 1:9. During such catalyzed stereoisomerization of the *cis* compound, a marked increase in the fluorescence (in ultraviolet light) became manifest, although this phenomenon was not as conspicuous as the analogous one observed upon the same treatment of *cis*–*trans*- or *cis*–*cis*-diphenylbutadiene.[5]

PASADENA, CALIF. RECEIVED JUNE 19, 1951

(6) A. Sandoval and L. Zechmeister in "Biochemical Preparations," Vol. I, John Wiley and Sons, Inc., New York, N. Y., and Chapman and Hall, Ltd., London, 1949, p. 57.