Summary.—Spores of the fungi *Rhizopus suinus*, *Mucor dispersus* and *Aspergillus melleus* were tested with respect to their resistance to ultraviolet of wave-length 2650 A. Sigmoid survival curves were obtained for the fungi *Rhizopus suinus* and the minus strain of *Mucor dispersus* when spores from 4-day cultures were irradiated. Survival curves describing a logarithmic order of death were exhibited by spores from 4-day cultures of *Aspergillus melleus*.

The three species of fungi showed marked differences in susceptibility to radiation (2650 A). A combination of the differences in pigmentation, size of the spores and the number of nuclei may account for the differences in susceptibility observed.

A trend of increasing resistance to radiation with increased age was observed for spores of *Rhizopus suinus*.

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PROLYCOPENE, A NATURALLY OCCURRING STEREOISOMER OF LYCOPENE

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In this paper we record the observation that there occurs in the variety of tomato called "tangerine tomato" a carotenoid, *prolycopene*, which is an isomer of lycopene; the isomeric relationship is similar to that between lycopene and neolycopene,[1] and in our opinion prolycopene is to be classed as a naturally occurring neolycopene, being the first observed natural *neo* form of a C_{40}-carotenoid.

The color of ripe tomato fruits is determined primarily by two sets

of genes, one of which, *Y* and *y*, affects the color of the skin, and the other, *R* and *r*, the color of the flesh.[2] The relation between these genes and the carotenoid pigments of the fruit has been studied.[3] In addition to the red genes *R* and *r* there is only one other pair of genes known which affects the flesh color of ripe tomato fruits.[4] This pair has been named "tangerine," *T* and *t*. In double recessive form *tt* it causes a brilliant orange color in the fruits which would have been red if the dominant gene *T* had been present. The two genes *R* and *T* are inherited independently and are located in chromosomes 2 and 7, respectively.

The tangerine tomatoes (a commercial variety) used in the present investigation were grown in a greenhouse kept at 26.5°C. during the day and 19°C. during the night. The conditions of temperature and humidity were the same as for red tomatoes studied earlier,[5] and the corresponding data of this paper and the earlier paper are directly comparable.

The tangerine tomato was mashed in a mortar and shaken with successive portions of methanol and petroleum ether (b. p. 60–70°) which were in such ratios that two liquid phases were present. The orange-yellow extract was washed free of methanol with water, dried over sodium sulfate, concentrated in vacuum and chromatographed on calcium hydroxide (Shell). The time required for the entire process up to the chromatographing was about two hours. By development with petroleum ether and benzene (1:1) or, better, with petroleum ether containing 10% acetone a chromatogram is obtained containing over a dozen layers (table 1). Lycopene is found near the top (layer 1), and neolycopene underneath it (layer 2). After the next minor pigment layers the main layer (layer 9), that of the new pigment prolycopene, appears, followed by several smaller layers and finally by β-carotene and its isomers (layers 13 to 15).

The main layer was eluted with petroleum ether and ethanol, washed free of ethanol, and dried. The solution was re-chromatographed after it had stood at 5° for one day. The new chromatogram showed about nine layers in addition to the main one. (Lycopene itself on similar isomerization gives a chromatogram with three preponderant layers.) These layers were similar to some of those (1 to 8, 10) in the original chromatogram, including layer 1, that of lycopene, which suggested that the new pigment had, on standing, in part isomerized to lycopene. A portion of a petroleum-ether solution of the main layer was then treated in the cell of a spectroscope (Evaluating Grating Spectroscope, Loewe-Schumm design, Carl Zeiss, Jena; 2-mm. Jena light filter No. BG-7) with an iodine solution (in petroleum ether), and the spectrum was observed to change very rapidly (within a second or two) from that of the original pigment (469, 441 mμ) to a more intense spectrum apparently identical with that of lycopene after similar treatment (502, 471, 441 mμ). The assumption that lycopene is the

preponderant component of the resulting pigment mixture was confirmed by adsorption analysis; the main layer of the chromatogram was lycopene,

TABLE 1

SPECTRA IN PETROLEUM-ETHER SOLUTION OF INDIVIDUAL PIGMENTS OF THE TANGERINE TOMATO, AND SPECTRA AFTER REACTION UNDER THE CATALYTIC INFLUENCE OF IODINE. THE PIGMENTS ARE IN THE SEQUENCE OBSERVED IN THE CALCIUM HYDROXIDE CHROMATOGRAM

LAYER NUMBER	SPECTRUM BEFORE CATALYSIS				SPECTRUM AFTER CATALYSIS			
1	505	475	446	(420)	502	471	441	(413) mμ
2	499.5	468	349		502	471	441.5	
3	498.5	468			501.5	471.5	441.5	
4	498.5	474.5	447		502	470.5	442	
5		475.5	445.5		502	471.5	441	
6		472	443.5	(417)	502	471	441.5	
7	497.5	469	438.5		502	471.5	440.5	
8	495.5	468	437.5		499	470.5	439.5	
9		468.5	441		502	470.5	441	
10		464.5	431		501	470.5	435.5	
11	(490.5)	(462.5)	430		(497)	470.5		
12		(Traces)				(Traces)		
13	486	455.5	427		485.5	456.5		
14	487	455			484	453.5		
15	481	450.5			483.5	452.5		

as verified by its spectrum (505, 475, 446 mμ) and by the mixed chromatogram method (a mixture of a solution of this layer and of a sample of pure lycopene was not separated in the chromatographic column). The other products of the catalytic isomerization are to be classed as neolycopenes.

The same spectral change was observed also to occur, more slowly than with iodine, under the catalytic influence of sulfur or hydrogen bromide in petroleum ether.

In another experiment the total extract (partially isomerized by standing) of the tangerine tomato was chromatographed, the individual layers were cut out and eluted, and their spectra in petroleum ether were determined. Then iodine was added to each solution and after a few minutes (at 30°) the spectra were again determined. The wave-lengths obtained in this experiment are given in table 1. It is seen to be probable that all of the pigments 2 to 11 are stereoisomers of lycopene; layer 2 is certainly the previously described neolycopene.[1]

Pending the establishment in detail of the stereochemical relations among the numerous isomers of lycopene, we have assigned the name prolycopene to the pigment described above, which is the main pigment of ripe tangerine tomato fruits. The results of our investigation suggest that the gene *R* may be involved in the synthesis of prolycopene in both tanger-

ine tomatoes and red tomatoes, and the gene *T* may then be responsible for the conversion of prolycopene into lycopene in red tomatoes.

The fact that the same catalysts are effective suggests that the relation between lycopene and prolycopene is similar to that between the carotenoids and their labile isomers, which have been under investigation recently.[6, 7, 1, 8, 9] Prolycopene has especial interest as the first naturally occurring C_{40}-carotenoid which gives rise to isomers which absorb light more intensely than itself and at longer wave-lengths. It is also interesting that the principal product of its isomerization is a well-known naturally occurring pigment.

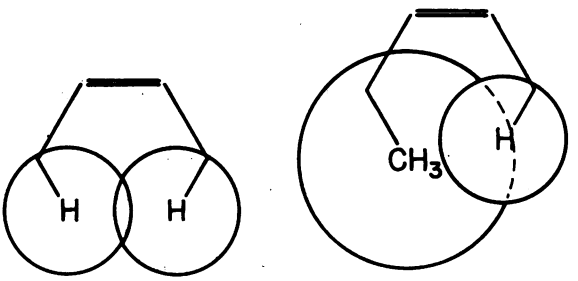


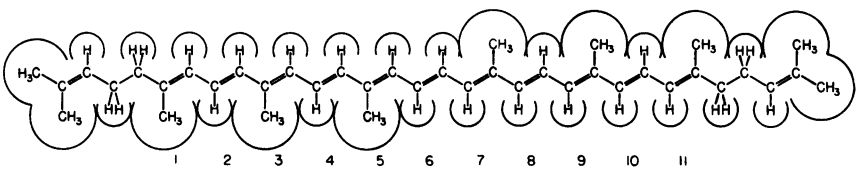
FIGURE 1

Drawings showing overlapping of hydrogen atoms in —CH—CR=CR—CH— and of hydrogen and methyl in —CH—CR=CR—CCH_3— with *cis* configuration.

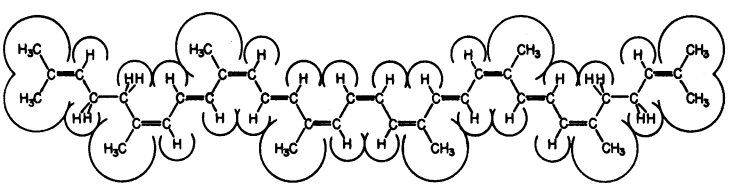
It now seems very probable that the suggestion[1] that the isomerization of the carotenoids is geometrical isomerization about the conjugated double bonds is essentially correct; the alternative suggestion[7] of double-bond migration must be abandoned as a general explanation because of the impossibility of accounting for the large number of the observed lycopene isomers on this basis. Experiments which are being carried on in these laboratories by Dr. A. Polgár have already shown that β-carotene also forms a larger number of isomers than can be explained by double-bond migration. We have, moreover, found that lutein, containing an α-ionone ring, is not converted into zeaxanthin, containing a corresponding β-ring, by treatment with iodine, which suggests that iodine is not effective as a catalyst for such double-bond migration.

Aside from the fact that the effective catalysts are known to catalyze *cis-trans* isomerizations, an argument based on the intensity of light absorption has been presented.[10] Another argument, as follows, may be based on the spectral shift, which is observed to be toward shorter wave-lengths for the *neo* forms. The theory of resonance requires that for effective conjugation the configuration about the single bonds as well as the double bonds of the system be coplanar.[11] The hydrogen atoms of CH

groups adjacent to a double bond with a coplanar *cis* configuration overlap somewhat, as shown in figure 1, where they are drawn with the normal van der Waals radius 1.0 A. (A smaller radius, 0.85 A, was used in drawing figure 10 of reference 10.) This overlapping and consequent interatomic repulsion would have two significant effects. First, it would decrease the stability of *neo* isomers (containing *cis* configurations) relative to the completely *trans* normal form, explaining the observation that in an equilibrium mixture the amount of the carotenoid possessing the spectrum with largest wave-length exceeds that of any one *neo* isomer.[12] Second, the repulsion would tend to push the *cis* molecule out of complete coplanarity, and thus to interfere to some extent with the conjugation and to shift the absorption maxima to shorter wave-lengths. Many *neo* isomers have maxima shifted by 5 to 7 mμ from those of the normal carotenoids; this we interpret as due to the interference by one *cis* bond with the conjugation. Zeaxanthin and capsanthin have also *neo* forms shifted by 10 or 12 mμ, and taraxanthin one shifted by 21 mμ, which we may interpret as resulting from two and three (or four) *cis* bonds, respectively.



LYCOPENE



PROLYCOPENE

FIGURE 2

Suggested stereochemical structures of lycopene and prolycopene.

The wave-length difference of 36 mμ between lycopene and prolycopene we would attribute to the presence in prolycopene of the *cis* configuration for as many as five or seven of the eleven conjugated double bonds. As shown in figure 1, steric interaction of hydrogen and methyl prevents the assumption of the *cis* configuration by double bonds with C—CH_3 adjacent;[10] i.e., those participating in the grouping C=C—C$\begin{matrix} \\ | \\ CH_3 \end{matrix}$, these being

the second, fourth, eighth and tenth double bonds of the series of eleven in the conjugated system of lycopene (Fig. 2). The suggestion advanced with some reserve by Karrer and Solmssen[13] that the double bond which differentiates labile and stable bixin is the third from the free carboxyl group (corresponding to number 8 of lycopene, figure 2) is incompatible with our arguments; this bond is of the type for which the *cis* configuration is forbidden.

It may be pointed out that not more than one double bond in each isoprene unit can assume the *cis* configuration, this being the bond adjacent to the methyl side chain. In addition, the central double bond (the one of β-carotene, for example, which is split during its conversion in the body into vitamin A) can also assume this configuration. The stereochemical structure suggested in figure 2 for prolycopene predicates that all seven double bonds for which this is possible are in the *cis* configuration, making prolycopene one extreme in the series of which the completely *trans* lycopene is the other. On isomerization prolycopene would not be converted directly into lycopene, but would pass through intermediate isomeric forms, *cis* about fewer than seven double bonds, as rotation occurred about the bonds one by one. The total number of possible *cis-trans* isomers for the seven stereochemically effective double bonds in the chain with equivalent ends is 72; in our small-scale chromatograms about a dozen zones have been observed, some of which may contain two or more components. Further support for the idea that prolycopene passes through intermediate *neo* forms during conversion into lycopene is given by the observation that the ratios of amounts of *neo* forms to lycopene in partially isomerized prolycopene are greater than for the mixture of isomers obtained by the isomerization of lycopene.

TABLE 2

NUMBERS OF *cis-trans* ISOMERS FOR UNSYMMETRICAL AND SYMMETRICAL CHAINS CONTAINING *n* STEREOCHEMICALLY EFFECTIVE DOUBLE BONDS

UNSYMMETRICAL CHAINS		SYMMETRICAL CHAINS	
$n = 1$	2 isomers	$n = 1$	2 isomers
2	4	2	3
3	8	3	6
4	16	4	10
5	32	5	20
6	64	6	36
7	128	7	72
8	256	8	136
9	512	9	272
10	1024	10	528
11	2048	11	1056
12	4096	12	2080

It is of interest in connection with our program of identifying the geometrical isomers of the carotenoids to consider the numbers of possible isomers. The chains are for this purpose divided into two classes: those, XX', whose two halves are not equivalent (called unsymmetrical chains) and those, XX, whose halves are topologically equivalent (called symmetrical chains). The formulas for the number of isomers for n effective double bonds (able to assume *cis* and *trans* configurations which are not equivalent) are the following: unsymmetrical chains, $N = 2^n$ for n odd or even; symmetrical chains, $N = 2^{(n-1)/2}(2^{(n-1)/2} + 1)$ for n odd, $N = 2^{(n/2)-1}(2^{n/2} + 1)$ for n even. Numerical values are given in table 2. It is seen that 10 isomers are expected for azafrin (unsymmetrical, $n = 4$); 32 for α-carotene, kryptoxanthin, lutein, capsanthin, semi-β-carotenone, citraurin and bixin (unsymmetrical, $n = 5$); 20 for β-carotene, zeaxanthin, astacin, capsorubin, rhodoxanthin, β-carotenone, norbixin, methylbixin and crocetin (symmetrical, $n = 5$); 64 for γ-carotene and rubixanthin (unsymmetrical, $n = 6$); 128 for lycoxanthin and rhodoviolascin (unsymmetrical, $n = 7$); and 72 for lycopene, as mentioned above (symmetrical, $n = 7$).

* Contribution No. 851.

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