benzfluorene. This is unusual, but a similar effect has been recorded by Bachmann and Carmack,[6] who compared the spectra of 3,4-benzpyrene and its 4′,5-dimethylene derivative.

The formulation of Bergmann and Eschinazi's hydrocarbon as a derivative of 3,4-benzfluorene requires the postulation of a five-membered ring closure, the product being 9,9-spirocyclohexanyl-3,4-benzfluorene, (VI)[1] in their article. The presence of the spirane ring should not affect the spectrum,[7] and the failure of this compound to hydrogenate further[1] also supports this formulation.

The spectrum of the quinone derived from (VI)[1] by oxidation, which has a probable structure (VII),[1] is recorded in Fig. 3; but in the absence of comparative data, this is of no value for the further characterization of the ring system.

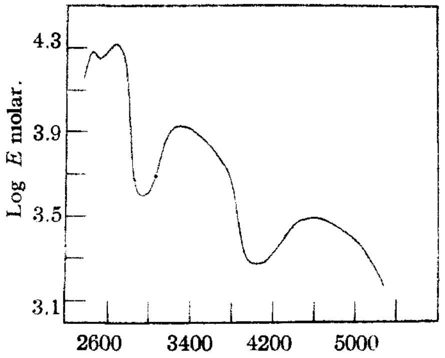

Fig. 3.——, Absorption spectrum of quinone (probably VII[1]).

TABLE I

POSITION AND INTENSITY OF ABSORPTION MAXIMA

Wave length, Å.	Intensity (log E_{molar})
(a) Hydrocarbon (VI)[1] (solvent ethanol)	
3150	4.45
3250	4.43
3395	4.55
3845	1.38
4105	1.61
4360	1.69
(b) Quinone (VII)[1] (solvent ethanol)	
2470	4.28
2680	4.32
3330	3.94
4600	3.49

In Table I data for the position and intensity of the maxima of the spectra of compounds (VI)[1] and (VII)[1] are recorded. The spectrographic methods employed have been reported previously.[8]

Acknowledgment.—The author wishes to express his thanks to Professor L. F. Fieser, of Harvard University, in whose laboratory the spectra were determined, and to acknowledge the financial support of The International Cancer Research Foundation.

Summary

Comparison of the ultraviolet absorption spectrum of a hydrocarbon synthesized recently by Bergmann and Eschinazi, with the spectra of chrysene, 3,4-benzphenanthrene and 3,4-benzfluorene suggests that the new hydrocarbon is 9,9-spirocyclohexanyl-3,4-benzfluorene.

KINGSTON, CANADA RECEIVED SEPTEMBER 10, 1943

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[6] Bachmann and Carmack, THIS JOURNAL, 63, 1685 (1941).

[7] Compare the spectra of 7,8-dihydrophenalyl-7-spirocyclopentane and naphthalene, in reference 5.

[8] Jones, THIS JOURNAL, 62, 148 (1940).

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY No. 939]

A Spectroscopic Study in the Stereoisomeric Capsanthin Set. *cis*-Peak Effect and Configuration

BY A. POLGÁR AND L. ZECHMEISTER

Recent experiments with some carotenoid hydrocarbons and alcohols have shown that the bending of the molecule by *trans* → *cis* rearrangements which modify the entire extinction curve produces a particularly characteristic change in a certain ultraviolet region of the spectrum.[1] This change which results in a new maximum, the "*cis*-peak," between 320 and 380 mμ has recently been given a theoretical interpretation.[2]

After a chromatographic separation of the main stereoisomers which are present in a mixture formed by iodine catalysis, the height of the *cis*-peak can be determined for each stereoisomer.[1,2,3] This procedure has been used recently to assign tentative configurations to the main observed members of the stereoisomeric sets of α-carotene, β-carotene, lycopene and lutein.[2,3]

In order to provide data for compounds of another type a carotenoid ketone, capsanthin, $C_{40}H_{58}O_3$, which is the main pigment of the red pepper (*Capsicum annuum*) has been investigated. Capsanthin was one of the first C_{40}-carotenoids in which stereochemical effects in the form of spontaneous isomerization were observed by Cholnoky and one of the authors.[4] This was

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[1] L. Zechmeister and A. Polgár, THIS JOURNAL, 65, 1522 (1943).

[2] L. Zechmeister, A. L. LeRosen, W. A. Schroeder, A. Polgár and L. Pauling, *ibid.*, 65, 1940 (1943).

[3] L. Zechmeister and A. Polgár, *ibid.*, 65, 137 (1943).

[4] L. Zechmeister and L. Cholnoky, *Ann.*, 530, 291 (1937).

followed by a more detailed study of the thermal and iodine catalyzed *cis–trans* isomerization of capsanthin, capsorubin, and some of their esters.[5]

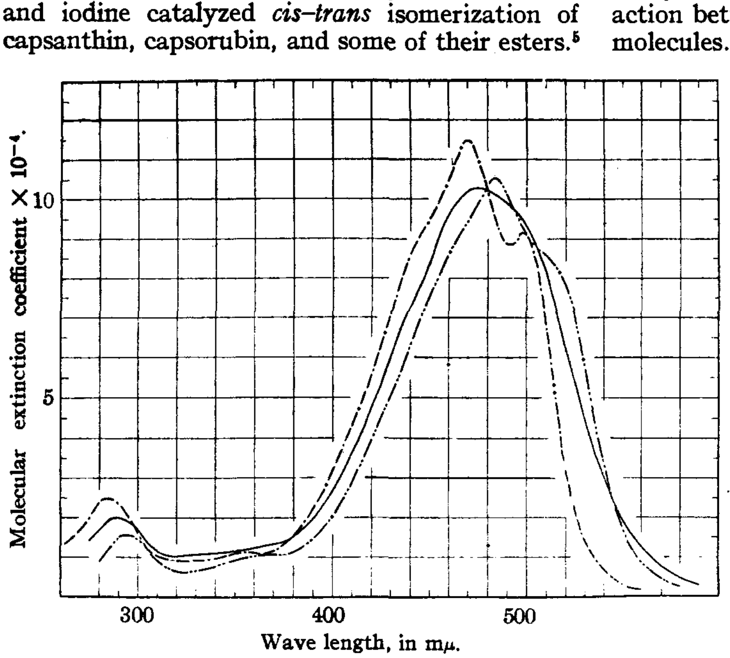

Fig. 1.—Molecular extinction curves of all-*trans*-capsanthin: —— in ethanol; -·-·- in benzene; and ----- in hexane.

Recent experiments show that capsanthin also undergoes a partial stereoisomerization upon melting its crystals or short exposure of solutions to sunshine.

We shall consider first the general shape of the capsanthin extinction curves, before and after stereoisomerization, and shall then discuss the possibilities for assigning spatial structures to some members of this set.

The extinction curve of natural (all-*trans*)-capsanthin in benzene was determined by Kuhn[6] in the visible region, and by Karrer and Würgler[7] also in the ultraviolet region; however, the latter paper is not available to us.

As one of our goals, we wished to obtain some data concerning the influence of polar solvents upon the shape of the extinction curve, especially upon the fine structure of the fundamental band in the visible region. It has been reported by Cholnoky and one of us[8] that the color of a solution of one milligram of capsanthin in two hundred milliliters of petroleum ether is straw-yellow, but that in alcohol is wine-red. Furthermore, the spectrum in alcohol is so blurred that it was impossible to locate bands with a visual spectroscope. Kuhn and Brockmann[9] noted earlier the same behavior in the polyene diketone, rhodoxanthin, $C_{40}H_{52}O_2$. They correctly attributed this phenomenon to the interaction between polar carbonyls and polar alcohol molecules. According to Kuhn and Brockmann, the effect is displayed only if the carbonyl group stands in conjugation with the carbon–carbon conjugated system. When this conjugation was interrupted by the addition of two hydrogen atoms to rhodoxanthin, the visual maxima in non-polar solvents (hexane) and in alcohol became identical in position and sharpness.

The very definite influence of the solvent upon the extinction curve of capsanthin may be seen by an inspection of Fig. 1. The complete lack of fine structure, which is probably caused by the presence of a number of pigment-alcohol complexes and the mutual superposition of their curves, explains our failure to read visual spectra in alcohol. An examination in hexane–alcohol mixtures proved that the fine structure of the fundamental band is markedly diminished even by small amounts of alcohol. As shown by Fig. 2 as little as 2% alcohol in hexane causes the greater part of the change from the 0% alcohol curve to the 100% alcohol curve.[9a]

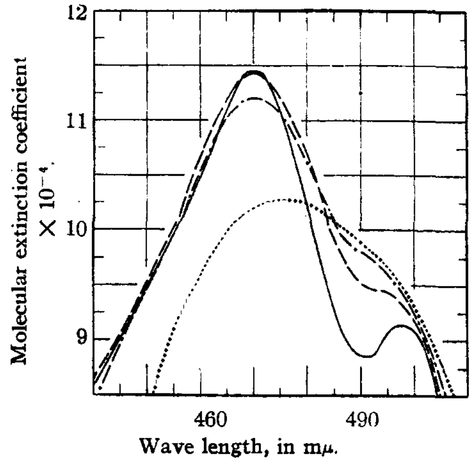

Fig. 2.—Molecular extinction curves of all-*trans*-capsanthin in the visible region: —— in hexane; - - - in hexane, containing 2% ethanol; -·-·- in hexane, containing 5% ethanol; and ..... in ethanol.

The stereochemical situation in the capsanthin set may be summarized as follows. The number of possible stereoisomers is thirty-two, and the number of the double bonds, which can undergo *trans* → *cis* isomerization, is five[10] (see the formula).

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(5) L. Zechmeister and L. Cholnoky, *Ann.*, **543**, 248 (1940).

(6) R. Kuhn, in L. Zechmeister, "Carotinoide," Julius Springer, Berlin, 1934, p. 235.

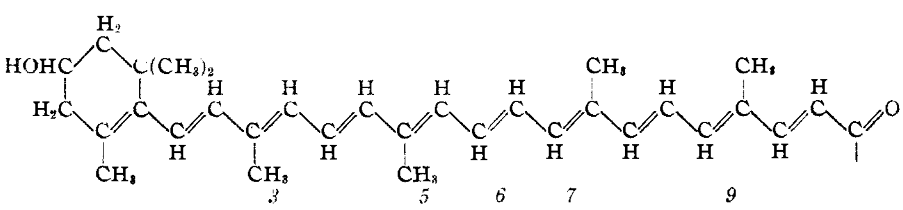
(7) P. Karrer and E. Würgler, *Helv. Chim. Acta*, **26**, 116 (1943).

(8) L. Zechmeister and L. Cholnoky, *Ann.*, **516**, 30 (1935).

(9) R. Kuhn and H. Brockmann, *Ber.*, **66**, 828 (1933); I. M. Heilbron and B. Lythgoe, *J. Chem. Soc.*, 1376 (1936) (with further references).

(9a) *Cf.* W. R. Brode, "Chemical Spectroscopy," 2nd ed., J. Wiley and Sons, Inc., New York, N. Y., 1943, pp. 185–188.

(10) L. Pauling, *Fortschr. Chem. organ. Naturstoffe*, **3**, 203 (1939); L. Zechmeister, A. L. LeRosen, F. W. Went and L. Pauling, *Proc. Natl. Acad. Sci.*, **27**, 468 (1941).



The chromophore of capsanthin
(The stereochemically effective double bonds are numbered)

It was shown in collaboration with Cholnoky[5] that capsanthin forms three main stereoisomers, the neo compounds A, B and C which are adsorbed above the all-*trans* pigment on the Tswett column. After catalysis with iodine for fifteen minutes the ratio, all-*trans*-capsanthin:neo A:neo B:neo C was 69:15:11:5, and fifteen hours later, 66:23:7:4.

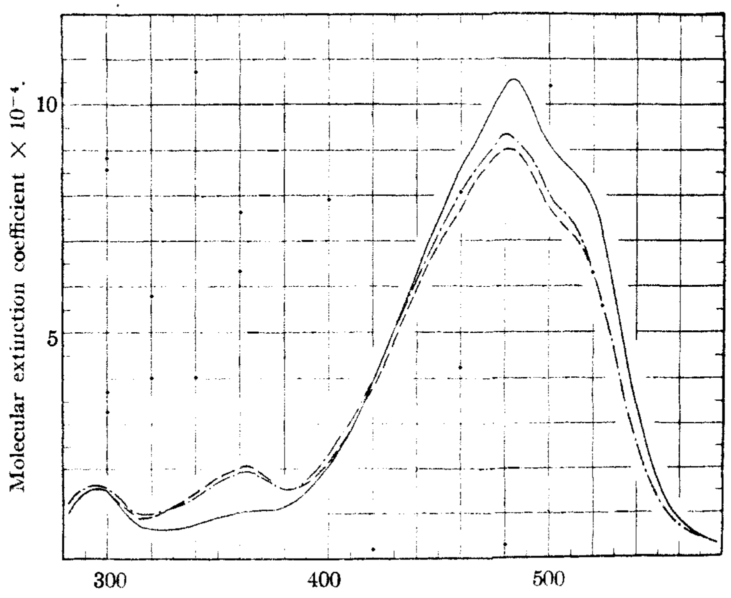


Fig. 3.—Molecular extinction curves of capsanthin in benzene: —— fresh solution of the all-*trans* compound; – – – mixture of stereoisomers after forty-five minutes of refluxing; and – · – · after iodine catalysis at room temperature, in light.

The extinction curves of fresh, refluxed and iodine catalyzed solutions of capsanthin show that the presence of a carbonyl group does not essentially alter the influence of stereoisomerization on the spectral properties. The extinction is diminished in the visible spectrum while in the ultraviolet region a considerable *cis*-peak appears, for example, in benzene at 363 mμ (Fig. 3).

Iodine does not cause the formation of a *cis*-peak in capsanthin solutions in the dark. Figure 4 shows, however, that an illumination as short as five seconds develops about a third of the ultimate effect. The photochemically excited iodine atoms are mainly responsible for this catalysis. As far as we know, the influence of light on the iodine catalysis of carotenoids is a general phenomenon. It has been briefly mentioned for the case of α- and β-carotene,[3] and we report below more on this subject. There are marked differences in the behavior of the individual carotenoids. Lycopene, for example, is considerably more sensitive than capsanthin. It was found by Dr. W. A. Schroeder in this Laboratory that a chromatogram of lycopene after an iodine treatment in the dark showed the presence of some isomers although the extent of isomerization was much less than in light. New experiments demonstrate that even in the dark a small *cis*-peak appears (Fig. 5) and nearly the total effect develops within five seconds when a solution of lycopene catalyzed with iodine is illuminated under conditions described in the Experimental Part.

The configurations of some neocapsanthins can be deduced tentatively from spectroscopic data. The longest wave length maximum of neocapsanthin A or B is shifted 6 mμ relative to that of the all-*trans* compound, and that of neo C is shifted 10.5 mμ. Our interpretation[2,3] demands that the molecules of neocapsanthin A and B contain one *cis* double bond but that the number of such bonds be two in neocapsanthin C.

Although the neocapsanthins A and B cannot be differentiated in the visual spectroscope, this is easily possible by a quantitative investigation of the *cis*-peak region. As shown in Fig. 6, the peak of neo A, which is comparable with the highest *cis*-peaks observed in the α- or β-carotene

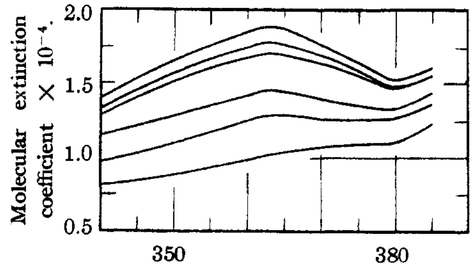


Fig. 4.—Influence of illumination on the development of the *cis*-peak effect in an iodine catalyzed solution of capsanthin, in benzene; curves from bottom to top: after 0 sec., 5 sec., 30 sec., 2 1/2 min., 15 min., and 30 min. illumination.

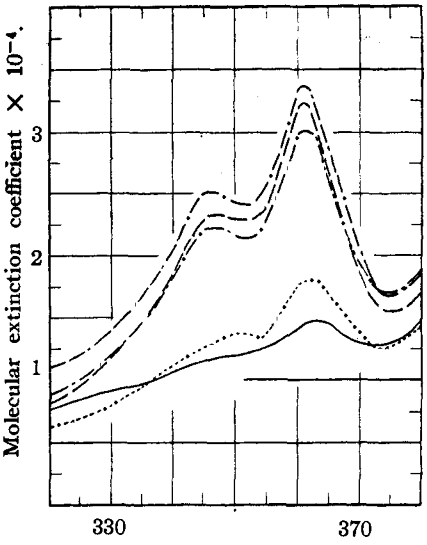


Fig. 5.—Influence of illumination on the development of the *cis*-peak effect in an iodine-catalyzed solution of lycopene, in hexane: ..... after 0 sec., – – – after 5 sec.; – – · – – after 30 sec., and –·–· after 15 min. illumination. The full line denotes the curve taken before the addition of iodine, and without illumination.

set, is much higher than the peak of neocapsanthin B. Consequently, and in accordance with recent investigations in other stereoisomeric sets, the most probable configuration of neocapsanthin A is that of *6*-mono-*cis*-capsanthin, and of neo B that of *5*-mono-*cis*- or *7*-mono-*cis*-capsanthin.[11] The position of the two *cis* double bonds in neo C cannot be stated definitely. The models do not exclude the possibility that one of these *cis* bonds is located at or near the center of the chromophoric system.

Acknowledgment.—We wish to thank Dr. L. Cholnoky for the preparation of the capsanthin samples.

Experimental Part

Materials and Methods.—The following solvents were used in the optical experiments: benzene (thiophene-free, reagent, Baker and Adamson), purified by shaking with sodium thiosulfate and then distilling; absolute ethanol, distilled over sodium; and hexane (practical, from petroleum, Eastman Kodak Co.), purified by shaking first with fuming sulfuric acid, then with alkaline permanganate, and finally redistilled.

The extinction readings were taken with a Beckman photoelectric spectrophotometer.[12] The extinction curves of all-*trans* pigments drawn in Figs. 1, 2 and 3 are based on average values (Tables I–II) of several independent experiments. The greatest deviation at the visual maxima is ±2.4% in benzene, and ±1.1% in ethanol. Because capsanthin is sparingly soluble in cold hexane, the solution was prepared by shaking crystals with this solvent and then filtering. The concentration was established by the use of extinction values obtained by dissolving capsanthin in absolute alcohol and diluting with 98 volumes of hexane; the maximum in Fig. 2 represented by a full line is, therefore, only approximately correct.

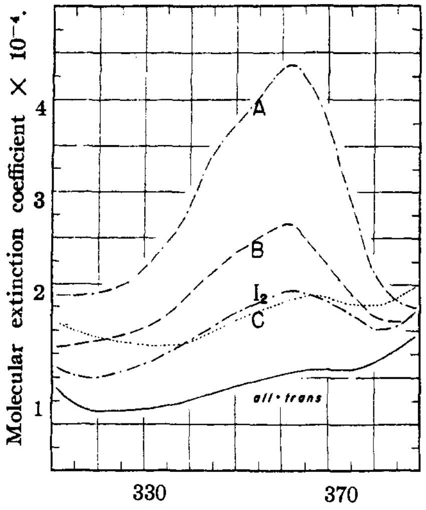


Fig. 6.—Molecular extinction curves of some members of the stereoisomeric capsanthin set in the *cis*-peak region, in benzene; I_2 indicates the equilibrium mixture obtained upon iodine catalysis.

Table I

Molecular Extinction Coefficients of Capsanthin and of its Stereoisomeric Equilibria at the Maxima (*italicized*) and Minima in Benzene

Fresh solution		After heat isomerization		After iodine isomerization	
mμ	$E^{mol.}_{1 cm.} \times 10^{-4}$	mμ	$E^{mol.}_{1 cm.} \times 10^{-4}$	mμ	$E^{mol.}_{1 cm.} \times 10^{-}$
508[a]	8.7	506[a]	7.4	506[a]	7.7
484	10.5	*481*	9.0	*481*	9.4
274–276[a]	1.08	382–385	1.52	382	1.52
		363	2.06	*362*	1.94
324–330	0.6(3)	315–318	0.9(1)	318	0.9(9)
295	1.56	*293–295*	1.56	*294*	1.63

[a] Point of inflection.

Table II

Molecular Extinction Coefficients of Capsanthin at the Maxima (*italicized*) and Minima in Hexane and Absolute Ethanol

In hexane		In ethanol	
mμ	$E^{mol.}_{1 cm.}$	mμ	$E^{mol.}_{1 cm.}$
498	9.1	*476*	10.3
492	8.8	320	1.04
468–470	11.4	*290*	2.00
365	1.08		
355	1.14		
320–330	0.9(2)		
284	2.50		

Influence of Illumination on the Development of the *cis*-Peak Effect in Solutions Catalyzed with Iodine.—Fresh solutions were prepared and catalyzed in the dark. After the extinctions had been determined, the solutions which were in 25-ml. volumetric flasks (glass), were placed in horizontal position on white paper and irradiated by means of two fluorescent lamps (3500° white, Mazda, 40 W; length of tube, 120 cm.) from a distance of 60 cm. The gradual development of the peaks of α-carotene, β-carotene, lycopene, and capsanthin is characterized by the data in Table III.

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(11) For nomenclature see reference 3.

(12) H. H. Cary and A. O. Beckman, *J. Optical Soc. Am.*, **31**, 682 (1941).

TABLE III

INFLUENCE OF LIGHT ON THE DEVELOPMENT OF THE *cis*-PEAK IN SOLUTIONS OF SOME CAROTENOIDS CATALYZED WITH IODINE

Duration of the illumination	Mol. extinction coeff. at the max. of longest wave length. $E^{mol.}_{1\,cm.} \times 10^{-4}$	Mol. extinction coeff. at the wave length of the *cis*-peak $E^{mol.}_{1\,cm.} \times 10^{-4}$	Increase in the *cis*-peak (% of the greatest change)
	α-Carotene (hexane)		
0 sec.	14.6	0.8(6)	0
5 sec.	14.2	1.51	45
30 sec.	13.7	1.94	75
2$^1/_2$ min.	13.4	2.11	87
15 min.	13.2	2.16	90
30 min.	12.7	2.30	100
60 min.	11.3	2.26	97
	β-Carotene (hexane)		
0 sec.	14.2	0.8(7)	0
5 sec.	13.9	1.49	40
30 sec.	13.2	1.95	69
2$^1/_2$ min.	13.0	2.04	75
15 min.	12.3	2.22	87
30 min.	10.9	2.22	87
60 min.	8.6	2.43	100
	Lycopene (hexane)		
0 sec.	18.6	1.47	0
5 sec.	15.5	3.2	92
30 sec.	15.6	3.4	100
2$^1/_2$ min.	15.4	3.2	92
15 min.	14.0	3.0	81
	Capsanthin (benzene)		
0 sec.	9.9	1.02	0
5 sec.	9.8	1.29	31
30 sec.	9.5	1.47	51
2$^1/_2$ min.	9.3	1.72	80
15 min.	9.0	1.80	89
30 min.	9.0	1.89	99
60 min.	8.9	1.90	100

TABLE IV

TYPICAL SPECTROSCOPIC DATA FOR SOME MEMBERS OF THE STEREOISOMERIC CAPSANTHIN SET (in BENZENE)

Member	Diff. between the visually established longest wave length maxima of the member and the all-*trans* form (mμ)	Mol. extinction coeff. at the *cis*-peak $E^{mol.}_{1\,cm.} \times 10^{-4}$	Diff. between mol. ext. coeff. of member and of all-*trans* form at *cis*-peak
Neo A	6	4.4	3.4
Neo B	6	2.65	1.60
Neo C	10.5	1.87	0.82
All-*trans*	0	1.05	0

In agreement with earlier data,[5] the visually observed maxima were 513, 481.5 mμ for the neocapsanthins A and B, and 508.5, 478.5 mμ for neo C, in benzene.

Estimation of *cis*-Peaks of the Neocapsanthins.—The mixture obtained from capsanthin by iodine catalysis in benzene was chromatographed on calcium carbonate (Merck precipitated Heavy Powder) and developed with benzene which contained 1 to 2% acetone. Each neo zone was eluted with ice-cold alcohol, transferred into benzene, washed thoroughly with ice water, dried with sodium sulfate, and investigated spectrophotometrically. The concentrations were established by catalyzing an aliquot part of each solution with iodine, determining the extinction at the wave length of the highest maximum, and calculating the concentration from the corresponding value of the iodine equilibrium curve.[1,2,3] Table IV contains the averages of two independent experiments.

***cis–trans* Isomerization of Capsanthin by Insolation.**—A solution of 2.5 mg. of all-*trans* pigment in 25 ml. of benzene was placed in a transparent quartz test-tube (diameter, 22 mm.) from which the air was displaced with carbon dioxide, and exposed to bright sunshine for thirty minutes (final temperature, 33.5°). After chromatography (23 × 4.8 cm.) and elution as described above, the colorimetric ratio of unchanged all-*trans* form to the neo capsanthins was 65:35. No appreciable quantity of irreversibly formed pigments appeared on the column.

***cis–trans* Isomerization of Capsanthin by Melting.**—Five milligrams of pigment, in a sealed tube, filled with carbon dioxide, was melted in a bath of 180° (5° above the melting point) for one minute. The color intensity decreased by about one-third. After cooling in ice-water, the benzene solution of the melt was chromatographed on calcium carbonate as described. The colorimetric ratio of unchanged all-*trans* pigment to the neocapsanthins was 29:71. The neo fraction contained some uninvestigated new isomers; furthermore, a cherry-red zone (3% of the total recovered pigment) appeared below that of all-*trans*-capsanthin. The chromatographic filtrate was slightly colored.

Summary

The interaction of the carbonyl group of capsanthin, $C_{40}H_{58}O_3$, with a polar solvent such as ethanol can be demonstrated by extinction curves. In contrast to the behavior in hexane, no fine structure of the fundamental band is observed in alcoholic solution. As little as 2% alcohol in hexane causes the greater part of the change from the 0% alcohol curve to the 100% alcohol curve. The modification of the extinction curve upon stereoisomerization is similar to that observed with carotenoid hydrocarbons and alcohols. Upon iodine catalysis a marked *cis*-peak develops at 363 mμ (in benzene) for which process light is needed. It is probable on the basis of spectroscopic data that neocapsanthin A contains one *cis* bond which is located at the center of the chromophore. This isomer should be assigned the configuration of *6*-mono-*cis*-capsanthin. Neocapsanthin B is probably either *5*-mono-*cis*- or *7*-mono-*cis*-capsanthin. Neocapsanthin C is a di-*cis*-compound.

PASADENA, CALIFORNIA RECEIVED SEPTEMBER 20, 1943