

acetylation. The over-all yield of lactose, based upon the acetobromo-D-galactose used in its synthesis, was 8%. The results constitute the structurally definitive syntheses of epi-lactose and lactose from D-mannose and D-galactose. They are total syntheses, since such syntheses of these hexoses were accomplished by Emil Fischer. The new

crystalline substances that are described are 2,3-diacetyl-4- $[\beta$ -2',3',4',6'-tetraacetyl-D-galactopyranosido]-D-mannosan <1,5> $\beta$ <1,6> and  $\alpha$ -octaacetyl-epi-lactose; some other new substances, intermediates in the syntheses, were not isolated in crystalline form.

BETHESDA, MARYLAND

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## Isomerization of $\beta$ -Carotene. Isolation of a Stereoisomer with Increased Adsorption Affinity

BY A. POLGÁR AND L. ZECHMEISTER

In spite of the fact that the phenomenon of carotenoid isomerization was first detected for  $\beta$ -carotene,<sup>1</sup> our knowledge in this special case is still very incomplete. These authors crystallized an isomer which they termed pseudo- $\alpha$ -carotene, and which appears immediately below  $\beta$ -carotene on the Tswett column. Gillam first assumed that the chromatographic process itself was responsible for the partial conversion, but it was shown later that the phenomenon occurs spontaneously and independently of a chromatographic experiment.<sup>2</sup> This conclusion was confirmed by Carter and Gillam.<sup>3</sup>

Early experiments in our laboratory showed that the reversible isomerization, for the explanation of which *trans-cis* shifts were suggested, can either increase or decrease the adsorptive power as compared with that of the respective all-*trans* carotenoid.<sup>4</sup> It was stated<sup>5</sup> as a rule that carotenoids with at least two free hydroxyl groups yield isomers which are adsorbed at higher sections of the column than the starting pigment. This result is in accordance with observations on the hydroxy-ketones capsanthine and capsorubine<sup>6</sup> as well as with investigations of Strain<sup>7</sup> concerning leaf xanthophylls. In the case of the monohydroxy-compound cryptoxanthin and especially of the hydrocarbons C<sub>40</sub>H<sub>56</sub> the opposite behavior was observed; to this rule, however, the present paper provides an exception.

(1) A. E. Gillam and M. S. El Ridi, *Biochem. J.*, **30**, 1935 (1936).

(2) L. Zechmeister and P. Tuzson, *ibid.*, **32**, 1305 (1938).

(3) G. P. Carter and A. E. Gillam, *ibid.*, **33**, 1325 (1939).

(4) L. Zechmeister and P. Tuzson, *Ber.*, **72**, 1340 (1939).

(5) L. Zechmeister, L. Cholnoky and A. Polgár, *ibid.*, **72**, 1678, 2039 (1939).

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

(7) H. H. Strain, "Leaf Xanthophylls," Carnegie Inst. Washington, No. 490, Washington (1938); *cf.* also F. W. Quackenbush, H. Steenbock and W. H. Peterson, *THIS JOURNAL*, **60**, 2937 (1938).

In systematic experiments now being carried out in our laboratory with a series of carotenoids mainly four methods of isomerization are applied, *viz.*, refluxing the pigment solution, iodine catalysis, hydrochloric acid catalysis (both at room temperature), and melting the crystals.<sup>8</sup> The first two procedures have already been described.<sup>2,4,5</sup> As a convenient method for the acid catalysis, a mechanical shaking of the petroleum ether solution with concentrated hydrochloric acid is used. The catalysis is here mainly effective at the continuously shifting intersurface of the two liquids.

It was assumed and confirmed by experiment that stereoisomers of a carotenoid which are formed and are present in the melt can be separated by rapid cooling and chromatography. Such a treatment, which is much milder than the so-called "thermal decomposition" of carotenoids,<sup>9</sup> is best carried out in a sealed tube, in the absence of oxygen. Under suitable conditions no carbonization occurs. The melting of  $\beta$ -carotene gave us four main types of products, *viz.*, unchanged starting material, reversibly formed stereoisomers of  $\beta$ -carotene, pigments with a much shorter chromophore than that of carotene, and finally colorless, strongly fluorescent substances with very low adsorption affinity. The ratio of these types depends on the conditions, especially on the temperature. A solution of a crude melt was found to show about one-half the initial color intensity, whereas "thermal decomposition" as practiced earlier leads to complete bleaching.

(8) It is intended to report on photochemical isomerization later.

(9) (a) J. F. B. van Hasselt, *Rec. trav. chim.*, **30**, 1 (1911); **33**, 192 (1914). (b) R. Kuhn and A. Winterstein, *Helv. chim. acta*, **11**, 427 (1928). *Ber.*, **65**, 1873 (1932); **66**, 429, 1733 (1933). L. Zechmeister and L. Cholnoky, *Ann.*, **478**, 95 (1930).

In the end-product of the heat, iodine, and melt isomerization the stereoisomers may be accompanied by minor pigments formed in an irreversible way. It is therefore important to test each zone of the chromatogram from this point of view. Only such pigments are qualified as stereoisomers of the starting material of which the solutions on addition of some iodine in the spectroscopic cell show a typical shift of the bands. The spectrum of an equilibrium mixture appears almost instantaneously in which the maxima are only a few millimicrons lower in wave length than those of the all-*trans* compound which was formed and predominates in the mixture. The result of the test may be confirmed by subsequent chromatography and the main zone of the chromatogram can be identified by means of a mixed chromatogram with a sample of the original carotenoid. The spectra of the chromatographic zones containing  $\beta$ -carotene stereoisomers are listed in Table I. All four isomerization methods yielded the pigments 1, 3, 5 and 8, while appreciable amounts of 2, 4 and 6 have been observed so far, mainly after melting or hydrochloric acid catalysis of  $\beta$ -carotene; 7 was obtained in a melt chromatogram of neo- $\beta$ -carotene U.

TABLE I  
SPECTRA OF REVERSIBLY FORMED STEREOISOMERS OF  $\beta$ -CAROTENE IN THE SEQUENCE OF DECREASING ADSORPTION AFFINITIES

No.	Extinction maxima ( $m\mu$ )		
1	481	450	(neo- $\beta$ -carotene U)
2	472.5	441.5	(neo- $\beta$ -carotene V)
3	486	454	( $\beta$ -carotene)
4	469	437.5	(neo- $\beta$ -carotene A)
5	475.5	444.5	(neo- $\beta$ -carotene B)
6	465.5	433	(neo- $\beta$ -carotene C)
7	474.5	441.5	(neo- $\beta$ -carotene D)
8	477.5	445	(neo- $\beta$ -carotene E)
9	468.5	437	} (No names given)
10	473.5	443	
11	476.5	445.5	
12	471	440	
13	473	444	

Pigment no. 5 is spectroscopically nearly identical with "pseudo- $\alpha$ -carotene."

Some uncertainty exists concerning the pigments 9-13 due to their great lability. In different experiments only one to two isomers appeared and could be differentiated below pigment 8. Because of this lability it was impossible to identify or differentiate two such zones originating from different kinds of experiments by mixed

chromatography. In this case the spectral data depend on the time which elapses between elution and optical measurement, and even on the rapidity of the reading, considering the photochemical effect of the light source. Above and including pigment 8 the individuality of the pigments listed is well established in spite of great differences in stability, because in all experiments, using the melt and the hydrochloric acid methods, a series of five to seven zones appeared in this section. Some members of this section must also be investigated rapidly; pigment 6 showed *e. g.*, 6  $m\mu$  longer wave length maxima than listed when in another experiment the time factor was not taken into consideration.

For one of the main stereoisomers of  $\beta$ -carotene which is adsorbed above  $\beta$ -carotene in the column, we suggest the name neo- $\beta$ -carotene U (U for ultra).<sup>10</sup> Depending on the conditions, 10-25% of  $\beta$ -carotene can be converted into this compound and 17% has been actually isolated in crystals.<sup>11</sup> The compound was also observed in extracts of pumpkin, squash, carrots, etc., where it was formed by spontaneous isomerization. A natural occurrence remains to be shown. The tendency for the formation of the U isomer is considerable and the fact that it was isolated only recently requires some explanation. While Gillam's pseudo- $\alpha$ -carotene (neo- $\beta$ -carotene B) appears below the usually sharp bottom line of unchanged  $\beta$ -carotene in the column and can easily be observed under various conditions, the U isomer (which is spectroscopically not very different from  $\beta$ -carotene) is included in the upper, more blurred part of the  $\beta$ -carotene zone and separates under adequate conditions only. With the brand of calcium hydroxide now in use in this laboratory, developing with petroleum ether which contains some acetone promotes the differentiation and finally produces a colorless interzone between the two stereoisomers.

The following remarks may summarize briefly our present views with regard to the stereochemistry of  $\beta$ -carotene.

(10) A final nomenclature cannot yet be given as the respective configurations must first be established (*cf.* footnote 12).

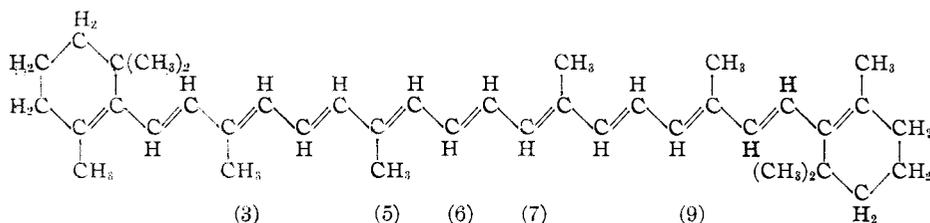
(11) Our results are in accordance with a short remark of H. H. Strain [THIS JOURNAL, 63, 3448 (1941)] that under the influence of acid "eschscholtzanthine, cryptoxanthine and  $\beta$ -carotene were converted slowly into substances that were adsorbed both above and below the native pigments." (In the iodine catalysis no pigment located above  $\beta$ -carotene in the column seems to have been observed.) In unpublished experiments we observed stereoisomers of  $\alpha$ -carotene which were adsorbed above the original pigment in the column. It is intended to report later on this subject.

According to Pauling<sup>12</sup> the steric interaction of hydrogen and methyl prevents the assumption of the *cis* configuration for double bonds with C-CH<sub>3</sub> adjacent. Therefore out of the eleven double bonds in  $\beta$ -carotene only five are stereochemically effective, *viz.*, numbers 3, 5, 6, 7 and 9 (see the formula).

The quantum mechanical treatment of the carotenoid spectra<sup>13</sup> leads to the conclusion that the greatest intensity of light absorption would be shown by the isomer with the all-*trans* configuration and that the shorter *cis* isomers would show decreased intensity. This is in agreement with our experiments, and with the *cis-trans* interpretation of the phenomena studied. Also, according to a private communication from Professor Linus Pauling who is working on the theoretical aspects of the problem discussed, the *cis* configuration for a double bond would be expected to produce a spectral shift in wave length toward the violet, with magnitude dependent on the position of the double bond in the long conjugated system.<sup>7</sup>

The following figures show that the spectral differences between the first maxima of all-*trans* polyene-hydrocarbons and their spectroscopically closest stereoisomers are about 4-6 m $\mu$ .

	m $\mu$		m $\mu$
$\beta$ -Carotene	486	$\gamma$ -Carotene	496
Neo- $\beta$ -carotene U	481	Neo- $\gamma$ -carotene A <sup>14</sup>	489.5
Lycopene		504.5 m $\mu$	
Neolycopene A		500.5 m $\mu$	



$\beta$ -Carotene (all-*trans*); the double bonds available for a *trans-cis* shift are numbered.

We tentatively assume with rough approximation that each *cis* double bond produces a spectral shift of the order of magnitude mentioned.

(12) (a) L. Pauling, *Fortschritte Chem. Organ. Naturstoffe*, **3**, 203 (1939); connected paper, (b) L. Zechmeister, A. L. LeRosen, F. W. Went and L. Pauling, *Proc. Nat. Acad. Sci.*, **27**, 468 (1941).

(13) L. Pauling, see footnote 12b, and *ibid.*, **25**, 577 (1939); R. S. Mulliken, *J. Chem. Phys.*, **7**, 364 (1939).

(14) The denomination "neo- $\gamma$ -carotene A" is now applied to that stereoisomer which appears immediately below unchanged  $\gamma$ -carotene on the Tswett column. Zones of the same pigment have been mentioned under the name "neo- $\gamma$ -carotene" by R. F. Hunter and A. D. Scott [*Biochem. J.*, **35**, 31 (1941)], and by the authors [*J. Biol. Chem.*, **133**, 193 (1941)]. Preliminary experiments, using refluxing and iodine catalysis, have revealed the presence of at least one more reversibly formed stereoisomer which is adsorbed below the A compound and is termed neo- $\gamma$ -carotene B.

The stereoisomer so far observed which shows the greatest displacement from the  $\beta$ -carotene maxima has bands at 465.5 and 433 m $\mu$ . The position of the first band ( $\Delta$  = about 20 m $\mu$ ) indicates the presence of four or five *cis* bonds, that is, all but one or all of the available double bonds have the *cis* form. This stereochemical situation is closely similar to that suggested for prolycopene and pro- $\gamma$ -carotene.<sup>15</sup> In the present case, however, its lability has prevented the crystallization of the substance.

Gillam's pseudo- $\alpha$ -carotene ( $\Delta$  = 9 m $\mu$ ) seems to contain two *cis* bonds while tentatively one such bond could be assigned to neo- $\beta$ -carotene U ( $\Delta$  = 5 m $\mu$ ).

Possibly neo- $\beta$ -carotene U contains in *cis* form that particular double bond (the central one) which on both sides is farthest from C-CH<sub>3</sub> groups and is attacked by the enzyme carotinase in the liver. It remains to be seen whether the biological formation of vitamin A is or is not preceded by a stereochemical shift. Neo- $\beta$ -carotene U has no vitamin A activity when tested with rats.

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### Experimental

**Methods.**—The pigment solutions were chromatographed on calcium hydroxide (Shell Brand lime, chemical hydrate; 98% through 325 mesh). For development petroleum ether (b. p. 60-70°) was used or, depending on the quality of the lime, the same solvent with 1-5% acetone. The figures on the left side of the described chromatograms denote width of the zones, in mm. Petroleum ether-alcohol mixtures are suitable eluents or else ether may be

(15) Footnote 12(b); A. L. LeRosen and L. Zechmeister, *This Journal*, **64**, 1075 (1942); L. Zechmeister and W. A. Schroeder, *ibid.*, **64**, 1173 (1942).

used if an immediate crystallization is intended. The spectra (in petroleum ether unless otherwise indicated) were determined with an Evaluating Grating Spectroscope (Zeiss, light filter BG-7, 2 mm. thick). For the estimation of the concentrations a Pulfrich Gradation Photometer was used (light filter S 45 or S 47). The necessary data for neo- $\beta$ -carotene U are given below. Values for  $\beta$ -carotene itself were published by Chohnoky.<sup>16</sup> Relative photometric values of components of a chromatogram are expressed in % of the extinction of the sum of the components.

(a) **Heat Isomerization of  $\beta$ -Carotene Solutions.**—A solution of 25 mg. of chromatographically homogeneous  $\beta$ -carotene crystals (from carrots) in 150 ml. of petroleum ether was refluxed in an all glass apparatus, in a slow carbon dioxide stream for 60 min. The following chromatogram was obtained (28  $\times$  7 cm.):

- 2 brownish yellow: irreversible layer (heterogeneous, about 485, 453  $m\mu$ .)
- 15 colorless
- 35 reddish orange: neo- $\beta$ -carotene U (481, 450)
- 2 almost colorless
- 0.5 yellow: irreversible layer<sup>17</sup> (479, 448)
- 2 almost colorless
- 85 dark orange:  $\beta$ -carotene (486, 454)
- 40 dark yellow: neo- $\beta$ -carotene B (475.5, 443.5)
- 2 almost colorless
- 20 pale reddish: neo- $\beta$ -carotene E (479.5, 447)
- 2 almost colorless
- 12 yellow: a labile isomer (473, 443)

Unchanged  $\beta$ -carotene and each stereoisomer (without irreversible zones) was cut out, transferred into petroleum ether and refluxed again. The relative photometric values of the zones thus formed are listed in Table II.

TABLE II  
RELATIVE PHOTOMETRIC VALUES OF THE PIGMENTS REVERSIBLY FORMED BY SIXTY MINUTES OF REFLUXING OF  $\beta$ -CAROTENE AND SOME OF ITS STEREOISOMERS

Starting material	Relative photometric values (%)				
	neo U	$\beta$ -carotene	neo B	neo E	labile isomer
Neo- $\beta$ -carotene U	31	40	19	10	
$\beta$ -Carotene	4	86	8	1	1
Neo- $\beta$ -carotene B	4	50	40	3	3
Neo- $\beta$ -carotene E	14	49	19	18	
Labile isomer	10	22	24	30	14

(b) **Isomerization of  $\beta$ -Carotene by Iodine Catalysis, at Room Temperature.**—25 mg. of homogeneous  $\beta$ -carotene in 200 ml. of petroleum ether was kept in the presence of 0.5 mg. of iodine for sixty minutes and chromatographed (calcium hydroxide, 28  $\times$  7 cm.). The same pigments as were listed in (a) appeared, except the 0.5-mm. yellow zone. Each zone was again submitted to this catalytic treatment with subsequent adsorption analysis. The rela-

(16) L. Chohnoky, *Z. Unters. Lebensm.*, **78**, 157 and 401 (1939); further communication in print.

(17) This minor layer has not been observed when stereoisomers of  $\beta$ -carotene were refluxed. If this pigment is refluxed separately, an irreversible pigment possessing 459, 430.5  $m\mu$  appears above it on the column. The same can be obtained from  $\beta$ -carotene by means of hydrochloric acid.

tive photometric values of the isomers formed are summarized in Table III.

TABLE III  
RELATIVE PHOTOMETRIC VALUES OF  $\beta$ -CAROTENE AND OF SOME OF ITS STEREOISOMERS AS FORMED BY IODINE CATALYSIS AT ROOM TEMPERATURE

Amount of iodine, 2% of the starting material; duration, 60 min.

Starting material	Relative photometric values (%)				
	neo U	$\beta$ -carotene	neo B	neo E	labile isomer
Neo- $\beta$ -carotene U	24	47	24	3	2
$\beta$ -Carotene	22	48	25	3	2
Neo- $\beta$ -carotene B	21	51	23	3	2
Neo- $\beta$ -carotene E	20	48	24	4	4
Labile isomer	18	45	16	13	8

(c) **Isomerization of  $\beta$ -Carotene with Hydrochloric Acid.**—25 mg. of homogeneous  $\beta$ -carotene in 200 ml. of petroleum ether was mechanically shaken with 100 ml. of concentrated hydrochloric acid for thirty minutes, then washed free of acid and developed with petroleum ether, containing 1% acetone, on a calcium hydroxide column (24  $\times$  5.5 cm.)

- 7 orange: irreversible layer (heterogeneous)
- 4 almost colorless
- 2 orange-yellow: irreversible (459, 430.5  $m\mu$ )
- 90 reddish orange: neo- $\beta$ -carotene U (480, 450)
- 5 almost colorless
- 40 dark orange:  $\beta$ -carotene (486, 454)
- 35 dark yellow: neo- $\beta$ -carotene B (476.5, 444)
- 12 pale reddish: neo- $\beta$ -carotene E (479.5, 447)
- 8 yellow: labile isomer (476.5, 445.5)
- 5 almost colorless
- 15 pale yellow: irreversible (479, 448.5)<sup>18</sup>

The relative photometric values of the zones were:  $\beta$ -carotene: neo U: neo B: neo E: labile isomer = 50:23:23:3:1. The chromatogram was subject to minor variations in different experiments. A reversible minor zone appeared on long development in most experiments below the U-isomer: neo- $\beta$ -carotene V (474, 443  $m\mu$ ), while in some other cases two isomers (471, 440 and 473, 444  $m\mu$ ) were observed below neo- $\beta$ -carotene E, both of which showed a very great tendency for spontaneous re-isomerization into  $\beta$ -carotene. If the indicated volume of acid was diminished or the duration of the catalysis shortened, the irreversible bottom layer was missing. In longer exposure to the acid, the amounts of the bottom layer increased; furthermore, two minor stereoisomers of  $\beta$ -carotene appeared, both located above the U-compound.

The irreversible bottom layer has been crystallized. A fresh solution of the crystals did not separate from carrot  $\alpha$ -carotene in the mixed chromatogram and will be investigated later.

(d) **Isomerization of  $\beta$ -Carotene by Melting.**—25 mg. of homogeneous  $\beta$ -carotene (m. p. 179.5°, cor.) crystals was melted in a sealed glass tube in carbon dioxide and kept in a bath at 190° for fifteen minutes. The melt was rapidly solidified in ice water, dissolved in petroleum ether and chromatographed. (The loss in the total photometric

(18) 0.5-1% of the starting material.

value of the starting material amounted to about 60%.) The column (27 × 6.8 cm.) was developed with petroleum ether containing 2% acetone:

- 15 colorless  
 1 yellow: irreversible (457.5, 427 m $\mu$ )  
 63 reddish orange: neo- $\beta$ -carotene U (481, 450)  
 2 pink: unidentified (very little)  
 7 yellow: neo- $\beta$ -carotene V (474, 443)  
 7 almost colorless  
 30 dark orange:  $\beta$ -carotene (486, 453.5)  
 2 almost colorless  
 15 dark yellow: neo- $\beta$ -carotene A (469, 437.5)  
 25 pale orange: neo- $\beta$ -carotene B (475.5, 444.5)  
 1 yellow: neo- $\beta$ -carotene C (471.5, 440.5)  
 3 colorless  
 15 pale reddish: neo- $\beta$ -carotene E (477.5, 445)  
 5 colorless  
 10 yellow: labile isomer (468.5, 437)

Filtrate: yellow (432 m $\mu$ ). This irreversible pigment (2% of the total photometric value of the melt) can be adsorbed on alumina (Alorco). The chromatographic filtrate of the latter contained a colorless substance showing greenish fluorescence in ultraviolet light.

The relative photometric intensities of the reversible zones (the width of which is printed in italics) were from top to bottom: 19:4:33:8:24:8:4.

(e) **Isolation and Properties of Neo- $\beta$ -carotene U.**—To a solution of 50 mg. chromatographically homogeneous  $\beta$ -carotene (from carrots) in 250 ml. of petroleum ether 1 mg. of iodine (in 1 ml. of the solvent) was added and the liquid kept at room temperature for an hour. The solution was then developed with petroleum ether containing 3–5% acetone on a calcium hydroxide column (28 × 7 cm.) until the following sequence appeared: near the middle of the column the typical deep orange zone of  $\beta$ -carotene was located (about half of the total pigment), and this was followed by a strong zone of neo- $\beta$ -carotene B and other minor neo-forms. Immediately above the  $\beta$ -carotene zone but well separated from it by a colorless intermediate section the light orange layer of neo- $\beta$ -carotene U was observed. The latter was cut out, eluted with alcohol and eventually combined with an analogous eluate obtained by a second treatment with iodine of the unchanged  $\beta$ -carotene portion. On addition of water the pigment was transferred into petroleum ether, washed free of alcohol, dried and re-chromatographed on a smaller column. Only minor zones appeared below the main product which was eluted with peroxide free ether. After evaporating the dried solution, the residue was dissolved in the minimum amount of benzene, transferred into a centrifuge tube and crystallized out by cautious addition of several volumes of absolute methanol. The crystallization was almost complete within a few minutes at room temperature and the yield was 12.7 mg., *i. e.*, about 25% of the starting material. If the second treatment of  $\beta$ -carotene is omitted, the yield is reduced by about one third (obtained 41 mg., from 250 mg. of  $\beta$ -carotene).

Neo- $\beta$ -carotene U was obtained as an orange crystalline powder which did not glitter. The basic microscopic form was a long narrow plate with one tapered end; the individuals were partly grouped in sheaves; m. p. 122–123°

(cor., electrically heated Berl block; sealed tube filled with carbon dioxide); a fraction isomerized during the determination.

*Anal.* Calcd. for C<sub>40</sub>H<sub>56</sub>: C, 89.48; H, 10.52; mol. wt., 537. Found: C, 89.34; H, 10.58; mol. wt., 486 (in exaltone; a considerable fraction was isomerized).

Neo- $\beta$ -carotene U is somewhat more soluble than  $\beta$ -carotene, especially in petroleum ether. It shows an epiphasic behavior in the partition test. The spectra, compared with those of  $\beta$ -carotene, are included in Table IV. On addition of iodine the following spectra appear: In carbon disulfide: 516, 481.5 m $\mu$ .; in pyridine: 498, 464.5 m $\mu$ .; in benzene: 495.5, 462 m $\mu$ .; in chloroform: 494, 461 m $\mu$ .; in carbon tetrachloride: 493.5, 461 m $\mu$ .; in dioxane: 490.5, 458 m $\mu$ .; in cyclohexane: 486.5, 455 m $\mu$ .; in acetone: 485, 453.5 m $\mu$ .; in ligroin (b. p. 86–100°): 484, 453 m $\mu$ .; in ethanol: 482, 450.5 m $\mu$ .; in petroleum ether (b. p. 60–70°): 485, 453 m $\mu$ .; in ether: 484, 452 m $\mu$ .; in hexane: 483.5, 452.5 m $\mu$ . and in methanol: (479), (448) m $\mu$ .

A 0.1% benzene solution did not show optical activity in a 2-dm. tube. The concentration of petroleum ether solutions can be determined by means of the Pulfrich Gradation Photometer ( $k$  = extinction coefficient,  $c$  = mg. of pigment in 100 ml. of solution).

	$k$	0.2	0.4	0.6	0.8	1.0
Light filter S45	$c$	0.085	0.18	0.27	0.36	0.45
Light filter S47	$c$	0.095	0.19	0.29	0.39	0.49

TABLE IV  
SPECTRA OF  $\beta$ -CAROTENE AND NEO- $\beta$ -CAROTENE U IN DIFFERENT SOLVENTS (m $\mu$ )<sup>a</sup>

Solvent	$\beta$ -Carotene	Neo- $\beta$ -carotene U
Carbon disulfide	520.5 484	512.5 478.5
Pyridine	503.5 469	498 464.5
Benzene	497.5 463.5	494 461
Chloroform	497.5 464	493.5 461
Carbon tetrachloride	497.5 463	492.5 460
Dioxane	494 462	490 458
Cyclohexane	490 457	485.5 454
Acetone	488.5 456	485 453.5
Ligroin (b. p. 86–100°)	487 455.5	483 452
Ethanol	486.5 453.5	482 450.5
Pet. ether (b. p. 60–70°)	486 453.5	481 450
Ether	485.5 453	481 449.5
Hexane	485 453.5	480 449.5
Methanol	484.5 452.5	480.5 449.5

<sup>a</sup> Gillam and El Ridi gave maxima for their pseudo- $\alpha$ -carotene in carbon disulfide: 507, 477 m $\mu$ .; in benzene, 491, 458 m $\mu$ .; in chloroform, 486, 456 m $\mu$ .; in ethanol, 478, 447 m $\mu$ .; and in petroleum ether (b. p. 70–80°), 477, 446 m $\mu$ .

Neo- $\beta$ -carotene U is less easily adsorbed on lime from petroleum ether than pro- $\gamma$ -carotene, much less than  $\gamma$ -carotene and considerably more strongly than  $\beta$ -carotene. The latter and neo- $\beta$ -carotene U can be separated by developing with petroleum ether but more easily when 2–5% acetone is added to the solvent, depending on the quality of the adsorbent. On some brands of calcium hydroxide no separation takes place from pure petroleum ether but it occurs in many cases in the presence of 15% benzene.

Isomerization experiments with different methods, which started from crystallized neo- $\beta$ -carotene U gave, with some variations, the stereoisomers mentioned above. The compound U is comparatively resistant to moderate heating, *e. g.*, a solution of 3 mg. in 25 ml. of petroleum ether, when kept at 60° for one hour, contained about three-fourths of the final pigment in the form of unchanged compound U. In melt experiments the same stereoisomers were obtained as in the corresponding experiments with  $\beta$ -carotene and a further reversible zone was observed (neo- $\beta$ -carotene D, 474.5, 441.5 m $\mu$ ). The melt was kept at 135° for fifteen minutes and because of the lower temperature (as compared with the  $\beta$ -carotene melt) and the partial formation of the more intensely colored  $\beta$ -carotene, the total loss in extinction was only one-fourth to one-fifth of the initial value in this case. Of the total photometric value of the melt 40% was caused by unchanged U-isomer, 22% by  $\beta$ -carotene formed and 38% by seven minor stereoisomers. No slightly colored or colorless and fluorescent breakdown products were observed.

### Summary

As a contribution to the stereochemistry of polyenes, the isomerization of  $\beta$ -carotene was

studied, under the influence of refluxing, iodine catalysis, hydrochloric acid catalysis, and by melting the crystals. The chromatograms showed a number of pigments, nine or ten of which were stereoisomers of  $\beta$ -carotene. Some of them are very labile. On addition of iodine they all yield a preponderant quantity of  $\beta$ -carotene and minor stereoisomers. One of the new isomers, "neo- $\beta$ -carotene U," has been crystallized in a yield of 17% of the starting material. It is adsorbed above  $\beta$ -carotene and thus does not follow an earlier suggested rule concerning the location of polyene-hydrocarbon isomers on the Tswett column. It is tentatively assumed that the U isomer contains one *cis* double bond, and Gillam's pseudo- $\alpha$ -carotene two such bonds, out of the five stereochemically available double bonds. An observed labile stereoisomer which shows spectral maxima at 20 m $\mu$ . shorter wave lengths than  $\beta$ -carotene may possess four or five *cis* bonds in its molecule.

PASADENA, CALIFORNIA

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## An Extension of the Acidity Scale

BY L. MICHAELIS AND S. GRANICK

After it had been recognized that the concentration of the hydrogen ion was only to a first approximation a useful concept for an acidity scale, *pH* was then defined as the logarithm of the reciprocal of the activity, instead of concentration, of the hydrogen ion. Because of the essential uncertainty involved in the definition of the activity of a single ionic species such as the hydrogen ion, one may avoid committing oneself to a definition in terms of strictly thermodynamical concepts and adopt the following working definition

$$pH = \frac{E - E_0}{2.3RT}$$

where *E* is the e. m. f. of a cell composed of a hydrogen electrode, the solution to be measured, a saturated potassium chloride bridge and a calomel cell. The constant *E*<sub>0</sub> is chosen either so that in a very dilute solution of hydrochloric acid (say 10<sup>-4</sup> *M*) *pH* equals the logarithm of the reciprocal of the concentration of the acid, or so that the *pH* in an equimolecular mixture of acetic acid and sodium acetate of infinitely low ionic strength equals the *pK* of acetic acid, whether this *pK* be determined by a thermodynamical or by a non-thermo-

dynamical method, such as the conductivity method.<sup>1</sup> These two ways of fixing the value of *E*<sub>0</sub> are, for all practical purposes, compatible with each other.

This working definition is valid only on the assumption that the liquid junction potential of the above galvanic cell is irrelevant, either because it is negligibly small, or because it is constant for such cases of *pH* determinations as practically may occur. This assumption breaks down for solutions of large ionic strength and especially for solutions of very high acidity or alkalinity. Here, the liquid junction potentials can by no means be said to be irrelevant. If one tries to establish a *pH* scale for extremely acid solutions, another working definition of the acidity scale has to be chosen, and, possibly, such that in the region of lower acidities it coincides with the ordinary *pH* scale. One approach to this problem has been made by Hammett,<sup>2</sup> who, by means of colori-

(1) One of the more recent presentations of this much discussed problem is given by MacInnes in "The Principles of Electrochemistry," Reinhold Publishing Corporation, New York, N. Y., 1939.

(2) L. P. Hammett, *Chem. Rev.*, **16**, 67 (1935); "Physical Organic Chemistry," McGraw-Hill Book Company, New York, N. Y., 1940.