

symmetry (*e. g.*, all *trans*-lycopene or prolycopene) this electronic transition gives rise to no effective dipole moment and hence to no *cis*-peak absorption. Other stereoisomers should show *cis*-peaks of varying intensity, the strongest being shown by the molecule which is *cis* about the central double bond. The *cis*-peak intensity should be roughly

proportional to the square of the distance between the center of the conjugated system and the midpoint of the straight line between its ends. By use of such considerations as these some observed stereoisomers of lycopene and γ -carotene are assigned definite spatial structures.

PASADENA, CALIFORNIA

RECEIVED JUNE 1, 1943

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY No. 930]

Conversion of Lutein in a Boric Acid-Naphthalene Melt. I

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Although a number of mild reagents or treatments can be used to alter the steric configuration of carotenoids, certain energetic reagents yield crystalline C_{40} -compounds with modified empirical formulas and structure. Since conversions of the latter type involve partial destruction, the conditions must be defined carefully in order to secure reproducible yields. A reaction of this type, the action of cold, concentrated hydriodic acid on carotenes, was described recently.¹

If crystals of chromatographically homogeneous lutein (xanthophyll, *ex Tagetes*), $HOC_{40}H_{54}OH$, are mixed with naphthalene (in order to depress the melting point), melted in the presence of fused boric acid, and then kept at 140° for a few minutes, a subsequent chromatogram shows many colored layers, none of which contains unaltered lutein. When lutein crystals alone are kept above their melting point for some minutes, stereoisomerization and partial bleaching take place with but negligible amounts of the compounds described in the present paper appearing. The yields are also low in lutein-naphthalene melts but they are substantially increased by the addition to the melt of boric acid or tetraboric acid or boric anhydride. Under the conditions described no analogous conversion of zeaxanthin took place.

The pigments contained in the three main chromatographic layers of the lutein conversion product were crystallized and found to contain

one oxygen atom only. Pending a final nomenclature, we designate them, in the sequence of decreasing adsorbability, as desoxyluteins I, II, and III. The yields, based on lutein = 100%, were: 3 to 4% for I, 10% for II, and 3 to 4% for III. The analytical estimations indicate the formula $C_{40}H_{56}O$ ($\neq H_2$) for all three compounds, which have the following structural features in

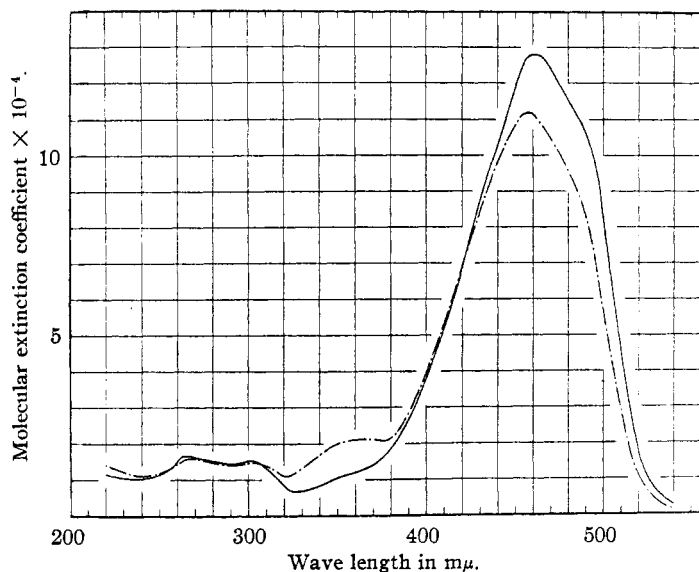


Fig. 1.—Molecular extinction curves of desoxylutein I in hexane: —, fresh solution of the all-*trans* compound; - - -, on iodine catalysis at 25° .

common: (1) as shown by negative biological assays for vitamin A activity in the rat, an unsubstituted β -ionone ring cannot be present, (2) catalytic hydrogenation indicates eleven double bonds, (3) the oxygen is present in the form of an esterifiable hydroxyl group; when partitioned between methanol and petroleum ether, the com-

(1) A. Polgár and L. Zechmeister, *THIS JOURNAL*, **65**, 1528 (1943).

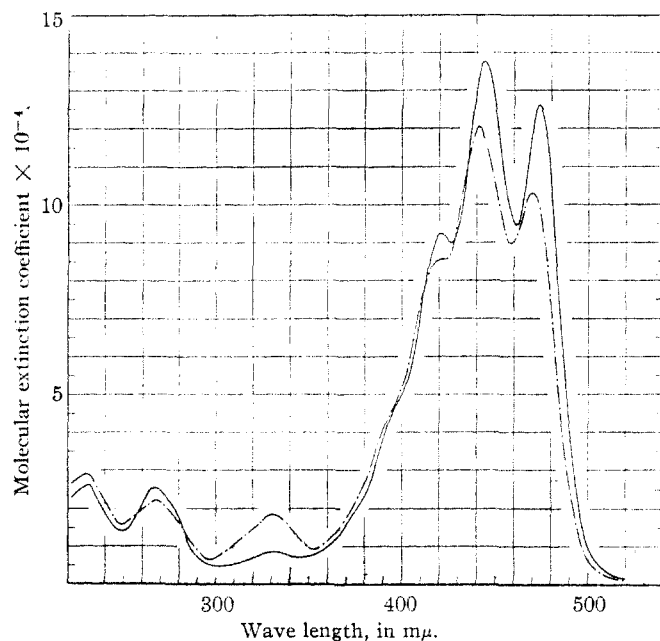


Fig. 2.—Molecular extinction curves of desoxylutein II in hexane: —, fresh solution of the all-*trans* compound; ---, on iodine catalysis at 25°.

pounds behave like cryptoxanthin, (4) the compounds are typical polyenes. On iodine catalysis they undergo *trans*-*cis* isomerization and develop *cis*-peaks² in the ultraviolet region (Figs. 1, 2 and 3).

Considering the great spectroscopic resemblance of desoxyluteins II and III to lutein in all wave length regions (Figs. 2 and 3), only ten of their eleven double bonds can be conjugated. Very different, however, is the spectrum of desoxylutein I (Fig. 1). While the extinction curves for II and III show the maxima and the fine structure characteristic for the fundamental band of lutein, compound I possesses only a single maximum in the visible region. The visually observed first "band" in petroleum ether is located at 494 $m\mu$. This position is identical with that of the first γ -carotene band and is about 17 $m\mu$ longer in wave length than the longest wave length maximum of desoxylutein II or III or lutein itself. The presence of a strengthened chromophore also becomes manifest on microscopic examination of the

(2) L. Zechmeister and A. Polgár, *THIS JOURNAL*, **65**, 1522 (1943); L. Zechmeister and W. A. Schroeder, *ibid.*, **65**, 1535 (1943); L. Zechmeister, A. L. LeRosen, W. A. Schroeder, A. Polgár and L. Pauling, *ibid.*, **65**, 1940 (1943).

crystals; I appears much redder than the brownish-orange crystals of II or III.

It is a reasonable assumption that the loss of one oxygen atom of lutein takes place by elimination of water in the boric acid melt, which may be accompanied or followed by other processes. The formation of the main product, *viz.*, desoxylutein II (or III) would be explained for example by the following sequence of re-

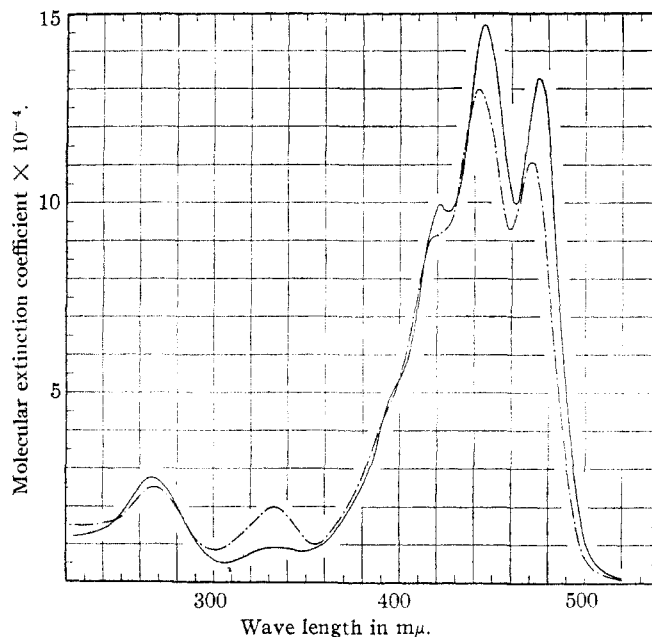
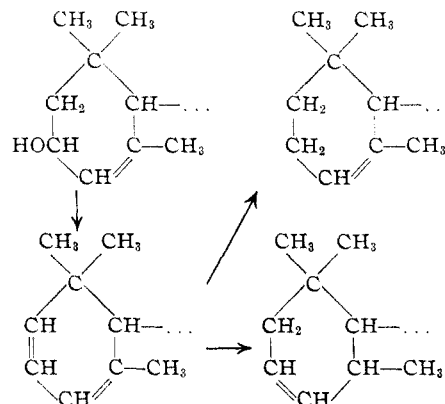


Fig. 3.—Molecular extinction curves of desoxylutein III in hexane: —, fresh solution of the all-*trans* compound, ---, on iodine catalysis at 25°.

actions which affect only the α -ionone ring of lutein.

Nothing conclusive can be put forward at this time for the structure of desoxylutein I, and particularly for the position of its powerful chromophore in the molecule. The fact that a very

blurred band appears in the visual spectroscop at a position identical with the longest wave length maximum of γ -carotene does not offer a sufficient spectroscopic basis for structural discussions. For example, a tentative identification of desoxylutein I with a hydroxy-dihydro- γ -carotene would not explain the lack of fine structure in the fundamental band, as represented in Fig. 1. The structural feature responsible for this phenomenon remains to be found.

It is as yet doubtful whether a relationship exists between the desoxyluteins and some pigments observed upon acid treatment of lutein under entirely different conditions.^{2a}

Acknowledgment.—We are indebted to Dr. C. E. P. Jeffreys for rat tests, to Mr. R. M. Lemmon and Mr. H. Pinckard for assistance in some experiments, and to Professor A. J. Haagen-Smit as well as Dr. G. Oppenheimer for microestimations.

Experimental

Preparation of Lutein.—The isolation of lutein from *Tagetes* extracts was first described by Kuhn, Winterstein and Lederer.³ A modified procedure making use of chromatography was employed in the following preparation.

One kg. of air-dried petals of *Tagetes erecta* (grown in Southern California) was ground and percolated with 5 liters of ether. The extract was saponified by standing over concd. methanolic potassium hydroxide overnight; then it was washed free from alkali in a continuous washing apparatus,⁴ dried with sodium sulfate and evaporated to dryness. The solution, prepared at 40°, of the residue in 450 ml. of benzene yielded crystals on addition of 3 vol. of petroleum ether (b. p. 60–70°) and standing at 5°. After recrystallization from chloroform-petroleum ether, the crude lutein crystals weighed 10 g. Their purification was carried out with 500-mg. portions. The solution of each portion in 100 ml. of benzene was developed with the same solvent on a calcium carbonate column (Heavy Powder, Merck, 30 × 8 cm.). The main zone was eluted with an ether-alcohol mixture, washed alcohol-free, dried with sodium sulfate and evaporated. This residue was crystallized from 25 to 30 ml. of chloroform by the addition of 4 vol. of petroleum ether. The yield of the pure lutein was approximately 165 mg. The glittering crystals were chromatographically homogeneous and did not separate in a mixed chromatogram from lutein obtained from another source. The analytical and optical data were correct.

Boric Acid Fusion.—One gram of pure lutein was divided into forty 25-mg. portions. Each portion, mixed

with 140 mg. of naphthalene and 60 mg. of finely-powdered, fused boric acid, was sealed in a Pyrex tube filled with carbon dioxide. The boric acid reagent had been obtained by melting boric acid (Reagent Crystals, Merck) at 250–275° for thirty minutes. (Boric anhydride, prepared by igniting boric acid over a Méker burner for forty-five minutes until a glass is formed, may also be used.)

Each tube was agitated in a dibutyl phthalate bath at 140° ($\pm 5^\circ$) for five minutes and then rapidly cooled in ice-water. The solution of the combined forty melts in 200 ml. of cold benzene was diluted with 4 vol. of petroleum ether and divided into eight parts, each of which was chromatographed on a column, 30 × 8 cm., prepared from a 1:1 mixture of calcium carbonate and calcium hydroxide (Shell Brand Chemical Hydrate, 98% through 325 mesh). Upon development with petroleum ether containing 5% acetone the following chromatogram appeared (the width of the zones in mm. is given on the left)

- 15 several minor zones: unidentified pigments
- 15 colorless
- 50 dark pink: desoxylutein I
- 40 yellowish-orange: desoxylutein II
- 4 dark pink: unidentified
- 6 brownish-orange: unidentified
- 40 yellow, with orange tint: desoxylutein III
- 20 yellow: unidentified

The chromatographic filtrate was yellow and showed spectral maxima at 469, 442.5 $m\mu$ in petroleum ether.

The respective zones of desoxylutein I, II, and III were treated in an identical manner. After elution with alcohol each pigment was transferred into petroleum ether by addition of water, washed alcohol-free, dried and developed with petroleum ether containing 5% acetone on calcium carbonate-hydroxide columns (30 × 8 cm.). Two such columns were needed for the rechromatography of I or III, and four for II. Desoxylutein I formed a 110-mm. pink zone, with a minor yellowish-orange layer immediately below it. The chromatograms of desoxylutein II showed a 30-mm. layer of desoxylutein I above the main 70-mm. yellowish-orange zone; several minor zones were located below it. The chromatogram of desoxylutein III was practically homogeneous and consisted of a 110-mm. pigment region.

After elution with alcohol, each of the three compounds was transferred into ether, washed, dried and evaporated at room temperature. Each residue was dissolved in a minimum amount of ether, transferred with a dropper into a 15-ml. centrifuge tube and evaporated to dryness. After the rapid dissolution of the residue in 0.5–1.0 ml. of benzene at 40–50°, 4–5 vol. of methanol was added with stirring at room temperature. Crystals appeared immediately. The liquid was kept at 5° overnight and centrifuged. The pigment was washed with 1 ml. of methanol, centrifuged, and recrystallized as before. When the methanol was added gradually over a period of several hours at room temperature, larger, glittering crystals were obtained.

While the isolation of compounds I and II never afforded any complications, it was difficult to obtain reliable separation of III from the bottom zone of the chromatogram. Crude samples of compound III were oily and had to be rechromatographed before they could be crystallized; even then the combustion data varied.

One gram of lutein yielded 38 mg. of desoxylutein I, 100

(2a) Cf. R. Kuhn, A. Winterstein and E. Lederer, *Z. physiol. Chem.*, **197**, 141 (1931); H. H. Strain, "Leaf Xanthophylls," Washington, 1937; F. W. Quackenbush, H. Steenbock and W. H. Peterson, *This Journal*, **60**, 2937 (1938).

(3) R. Kuhn, A. Winterstein and E. Lederer, *Z. physiol. Chem.*, **197**, 141 (1931).

(4) A. L. LeRosen, *Ind. Eng. Chem., Anal. Ed.*, **14**, 165 (1942).

mg. of desoxylutein II, and 43 mg. of desoxylutein III. In a similar naphthalene melt with boric anhydride the respective yields were 30, 127 and 32 mg.

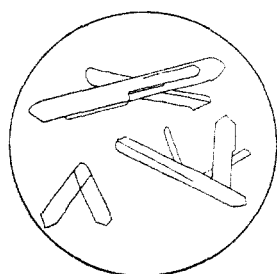


Fig. 4.—Desoxylutein I, crystallized from benzene and methanol.

Desoxylutein I.—This compound formed long prisms with peaked ends, partially grouped in aggregates (Fig. 4). Their color under the microscope was a rich reddish-orange which changed to brownish-red at crossings (compounds II and III appeared yellow or brownish in comparison); m. p. 149° (cor., in a sealed tube filled with carbon dioxide, in an electrically heated Berl block).

Anal. Calculated for $C_{40}H_{56}O$: C, 86.89; H, 10.22. Found: C, 87.09; H, 10.33. C, 86.29; H, 9.76. C, 86.59; H, 9.57. (These data are corrected for 1.6, 1.5 and 0.5% ash, respectively.) *Molecular weight.* Calcd. for $C_{40}H_{56}O$: 553. Found (in camphor): 524. *Catalytic Hydrogenation.*⁵—9.730 mg. of substance in methylcyclohexane and glacial acetic acid in the presence of 9.73 mg. of PtO_2 added 4.70 ml. of hydrogen (over mercury; 21.5°, 743.5 mm.); 4.729 mg. with 2.23 mg. of the catalyst added 2.33 ml. (22.5°, 739.5 mm.). Found: 10.8 and 10.9 double bonds.

The compound is easily soluble in cold benzene, ether and chloroform. It is much less soluble in petroleum ether, in which the crystals dissolve slowly. The solubility in methanol is very slight. On partition between petroleum ether and 85% methanol the pigment showed a purely epiphasic behavior, while a portion migrated into the lower phase when 95% methanol was employed.

In accordance with the extinction curve⁶ (Fig. 1) the visually-observed spectrum (in a Loewe-Schumann Grating Spectroscope, Zeiss) of desoxylutein I has an essentially different character from those of II or III or of lutein itself. The limits of the bands are indistinct and the interspace is never clear. In petroleum ether (b. p. 60–70°) only at very low concentrations can a main blurred band be noticed, *viz.*, around 458–459 $m\mu$, and a much weaker one at approximately 494 $m\mu$. Even a small increase in the concentration, however, brings about an extended shadowed area, located between 450 and 550 $m\mu$. In benzene solution the bands are even more blurred and unreadable. In carbon disulfide the main maxima were found near 537 and 493 $m\mu$.

Upon the addition of iodine the spectrum blurs and migrates toward shorter wave lengths. The maxima of the equilibrium mixture in petroleum ether are located near 491.5, 459.5 $m\mu$.

On calcium carbonate–calcium hydroxide (1:1) desoxylutein I is adsorbed below gazanixanthin (a hydroxy- γ -

carotene),⁷ lutein or lycopene when developed with petroleum ether containing 5 to 10% acetone. Under similar conditions desoxylutein I is adsorbed above cryptoxanthin.

Isomerization by Iodine Catalysis.—A solution of 1 mg. of chromatographically homogeneous desoxylutein I in a few drops of benzene was diluted to 15 ml. with petroleum ether, treated with a little iodine solution (10 to 20 μ g.) and chromatographed on a calcium hydroxide–carbonate column (1:1; 17 \times 1.9 cm.) after twenty minutes of standing at 25°. On development with petroleum ether containing 5% acetone, a new zone appeared below the unchanged portion of the all-*trans* form. The neo-compound showed blurred maxima at 486.5, 452 $m\mu$ (in petroleum ether) which changed to 489.5, 456.5 $m\mu$ on addition of iodine. That the all-*trans*- and the neo-compounds give identical equilibrium mixtures with iodine was shown by chromatography (*cf.* also Fig. 1).

Acetate of Desoxylutein I.—5 mg. of substance in 1.5 ml. of anhydrous pyridine was treated at room temperature with 0.5 ml. of acetic anhydride for twenty-four hours. The pigment was then transferred with water into petroleum ether solution which was washed, dried and chromatographed on a calcium hydroxide–carbonate (1:1) column (20 \times 3.8 cm.). Development with petroleum ether containing 5% acetone gave two zones, the lower one of which contained the main portion of pigment in the form of the acetate. The eluate of the latter was transferred into ether, evaporated and the dry residue was recrystallized from benzene–methanol as small plates, m. p. 139° (cor.). The observed visual spectra did not differ from those of the unesterified compound; the partition behavior, however, was purely epiphasic. Saponification of the acetate in petroleum ether with cold methanolic potassium hydroxide yielded a chromatographically homogeneous pigment which did not separate from desoxylutein I on the Tswett column.

Desoxylutein II.—The characteristic crystal forms of this compound are represented in Fig. 5. The color of the crystals resembles that of lutein, both macro- and microscopically; m. p. 156.5–158° (cor., after some softening).

Anal. Calcd. for $C_{40}H_{56}O$: C, 86.89; H, 10.22. Found: C, 86.73; H, 9.93. C, 86.78; H, 10.12. C, 86.47; H, 9.82. The data are corrected for 0.5, 0.5 and 0.4% ash, respectively. *Molecular weight.*—Calcd. for $C_{40}H_{56}O$: 553. Found (in exaltone): 569 and 577.

Catalytic hydrogenation.—10.284 mg. of substance added in the presence of 5.31 mg. of PtO_2 , 5.09 ml. of hydrogen (22.5°, 742.0 mm.); 6.369 mg. with 2.41 mg. of catalyst added 3.05 ml. (22.5°, 741.5 mm.). Found: 11.0 and 10.7 double bonds.

The solubility and the partition behavior are essentially the same as in the case of desoxylutein I.

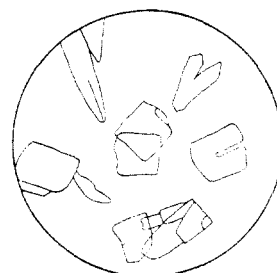


Fig. 5.—Desoxylutein II, crystallized from benzene and methanol.

(5) These estimations were carried out in the apparatus devised by A. N. Prater and A. J. Haagen-Smit, *Ind. Eng. Chem., Anal. Ed.*, **12**, 704 (1940).

(6) All extinction curves were taken in a Beckman photoelectric spectrophotometer: H. H. Cary and A. O. Beckman, *J. Optical Soc. Am.*, **31**, 682 (1941).

(7) K. Schön, *Biochem. J.*, **32**, 1506 (1938); L. Zechmeister and W. A. Schroeder, *This Journal*, **65**, 1535 (1943).

The following spectral maxima were established visually (the figures given in parentheses were taken after the addition of iodine): in carbon disulfide, 508.5, 473.5, 444 $m\mu$ (505.5, 471.5 $m\mu$); in benzene, 490.5, 458 $m\mu$ (488, 456 $m\mu$); in petroleum ether, 477, 446.5 $m\mu$ (475, 444 $m\mu$); in hexane, 476.5, 446 $m\mu$ (473.5, 443 $m\mu$); and in ethanol, 480, 449 $m\mu$.

When developed with petroleum ether containing 5–10% acetone, on a calcium hydroxide-carbonate (1:1) column, the compound is adsorbed below lutein, lycopene, desoxylutein I, and cryptoxanthin but above β -carotene and desoxylutein III.

Isomerization by Iodine Catalysis.—In an experiment similar to that carried out with desoxylutein I two stereoisomers were observed below the unchanged portion of the all-*trans* form. The spectral maxima were at 468.5, 439.5 $m\mu$ and 467, 438 $m\mu$ for the upper and lower neo-compounds, respectively. Both stereoisomers as well as the all-*trans* pigment shifted their maxima to 473.5, 443.5 $m\mu$ on iodine catalysis in petroleum ether solution (*cf.* Fig. 2). The three mixtures of stereoisomers obtained in this manner gave practically identical chromatograms.

Acetate of Desoxylutein II.—This ester was prepared as described for compound I. The crystals form plates, most of which have curved sides; m. p. 141° (cor. after softening at 139°). The compound shows a purely epiphasic behavior. The spectral maxima were not changed either by the acetylation or the saponification of the ester. The saponified pigment was found identical with desoxylutein II in a mixed chromatogram.

Desoxylutein III.—The crystals viewed under the microscope show a typical barrel-like or boat-like shape (Fig. 6). Their color is similar to that of compound II; m. p. 162° (cor., after softening). The samples were free of ash.

Anal. Calcd. for $C_{40}H_{56}O$: C, 86.89; H, 10.22; for $C_{40}H_{54}O$: C, 87.24; H, 9.88. Found: C, 86.92; H, 9.74. C, 87.19; H, 10.13. (In unfavorable cases mentioned above the carbon values were markedly different, *e. g.*, 87.75 and 85.96%.) **Molecular weight.** Calcd. for $C_{40}H_{56}O$: 553. Found (in exaltone): 547. **Catalytic hydrogenation.** 9.300 mg. of substance with 4.49 mg. of PtO_2 added 4.61 ml. of hydrogen (22°, 740.5 mm.); 7.774 mg. with 3.49 mg. of the catalyst added 3.72 ml. (23°, 742.0 mm.). Found: 11.0 and 10.7 double bonds.

The visually-observed spectral maxima follow (the figures in parentheses were taken after iodine catalysis): in carbon disulfide, 508.5, 474 $m\mu$ (506, 472 $m\mu$); in ben-

zene, 491, 458.5 $m\mu$ (489.5, 456.5 $m\mu$); in petroleum ether, 478, 448 $m\mu$ (476, 445 $m\mu$); in hexane, 477.5, 447 $m\mu$ (475.5, 445 $m\mu$); and in ethanol, 481.5, 450.5 $m\mu$ (with iodine, blurred and shifted toward shorter wave lengths).

Desoxylutein III is adsorbed below II and very slightly below γ -carotene when developed on a calcium carbonate-hydroxide mixture (1:1) with petroleum ether containing 2.5 to 5% acetone. β -Carotene occupied a place considerably below III under similar conditions.

Isomerization by Iodine Catalysis.—The chromatogram of the catalyzed solution showed, in addition to unchanged all-*trans* pigment and a minor, irreversible top layer, a neo-compound adsorbed below the main zone. Its spectral maxima in petroleum ether were: 468, 429 $m\mu$ (with iodine, 474.5, 443.5 $m\mu$). The steric change is reversible as shown by chromatography of the mixture obtained from the neo-compound with iodine.

A crystalline acetate of compound III has not yet been obtained.

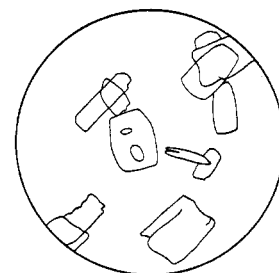


Fig. 6.—Desoxylutein III, crystallized from benzene and methanol.

Summary

Lutein (*ex Tagetes*), $C_{40}H_{56}O_2$, when melted with boric acid in the presence of naphthalene, gives rise to a mixture of new polyenes which can be separated chromatographically. The three main conversion products, each containing one oxygen atom, were crystallized and tentatively termed desoxyluteins I, II, and III. Their main characteristics are described and some structural features are discussed. The extinction curve of I shows only one maximum in the visible region while the fundamental band of II or III has the fine structure of the lutein curve. On iodine catalysis *trans-cis* changes occur and a "*cis*-peak" appears in all three cases.

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RECEIVED JUNE 8, 1943