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cis–trans Isomerization and Spectral Characteristics of Gazaniaxanthin. Further Evidence of its Structure

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In a series of investigations on γ-carotene and its derivatives, it has been desirable to study a monohydroxy-γ-carotene from the standpoint of its *trans–cis* isomerization.

The first hydroxy-γ-carotene, rubixanthin, $C_{40}H_{55}OH$, was isolated from rose hips ("Hagebutten," *Rosa canina*, *R. rubiginosa*, etc.) by Kuhn and Grundmann.[2] They proved that the hydroxyl group is located on the β-ionone ring while the acyclic end terminates in an isopropylidene group. Because the plant material required for the isolation of rubixanthin was not available, we have studied the behavior of the related carotenoid, gazaniaxanthin.

This carotenoid is the main component of the polyene pigment of the flowers of *Gazania rigens*, R. Br. (*Compositae*) in which it was detected by Schön.[3] Our starting material, grown in Southern California, contained the same gazaniaxanthin but the pigment mixture was markedly different from that described by this investigator. The variation is understandable when the difference in geographical location is considered (Schön worked in Portugal). Table I presents a comparison of the respective mixtures.

TABLE I

MAIN COMPONENTS OF THE CAROTENOID PIGMENT IN *Gazania rigens* FLOWERS[a]

Grown in Portugal	Grown in Southern California
Lutein	Lutein
Rubixanthin	*Gazaniaxanthin*, 1400 mg./kilo
Gazaniaxanthin	*Lycopene*, 435 mg.
Unknown[b]	*Cryptoxanthin*
γ-Carotene	γ-*Carotene*, 100 mg.
β-Carotene	β-Carotene, 60 mg.

[a] The italicized compounds have been isolated as crystals. The figures refer to the content per kilo of dry flowers. The yields were 30–45% of the gazaniaxanthin, 30% of the lycopene, and 13% of the γ-carotene content listed.

[b] From Schön's description of this fraction, it is not unreasonable to assume that this was a mixture of neogazaniaxanthins. The spectrum, identical with that of γ-carotene, may have been formed by re-isomerization.

In his important paper, Schön not only described the isolation of gazaniaxanthin, but also suggested that it may be a monohydroxy-γ-carotene having the formula $C_{40}H_{54}O$ or $C_{40}H_{56}O$. He demonstrated the presence of a hydroxyl group by the synthesis of a crystalline acetate which we have confirmed by the determination of one active hydrogen according to the method of Zerewitinoff. Schön postulated that the hydroxyl group might be located "in the aliphatic side-chain, like in lycoxanthin and lycophyll"[4] but he rightly pointed out that such an assumption should be tested by a biological assay. Such an assay has now been carried out. Because our sample of gazaniaxanthin (like rubixanthin[2]) proved to be inactive as a provitamin A in the rat, the hydroxyl group must be located on the β-ionone ring.

Analytical investigations to elucidate further details of the structure have led to contradictory results. An hydroxy-γ-carotene would possess twelve double bonds but catalytic hydrogenation has proved the presence of only eleven in gazaniaxanthin. These must be conjugated in order to produce the spectrum observed. This result would indicate that the molecule does not contain an isolated double bond and consequently that the empirical formula is $C_{40}H_{58}O$. However, ozonolysis indicated the presence of one isopropylidene group.[5] Since compounds such as thymol give 0.3 "isopropylidene" group per mole, it may well be that some carotenoids containing an isopropyl group would also yield acetone on ozonolysis. Therefore, gazaniaxanthin may be a dihydrorubixanthin as expressed below in a tentative formula.

Fresh, dilute solutions of gazaniaxanthin are relatively stable at room temperature. In twenty-four hours only 4% of the pigment underwent *trans–cis* isomerization in petroleum ether, while upon refluxing in benzene for fifteen minutes, an equilibrium was reached which contained about

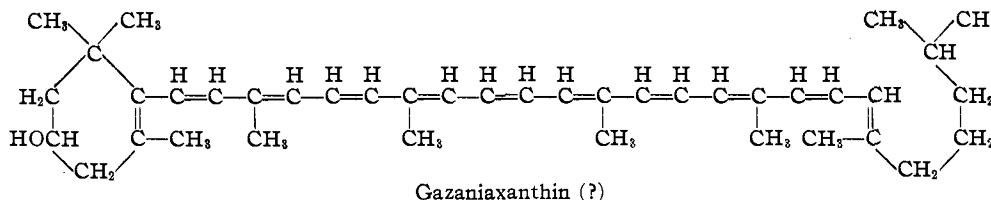
(1) Allied Chemical and Dye Corp. Fellow.

(2) R. Kuhn and Ch. Grundmann, *Ber.*, **67**, 339 and 1133 (1934).

(3) K. Schön, *Biochem. J.*, **32**, 1566 (1938).

(4) L. Zechmeister and L. Cholnoky, *Ber.*, **69**, 422 (1936).

(5) R. Kuhn and H. Roth, *ibid.*, **65**, 1285 (1932); F. Pregl and H. Roth, "Quantitative Organic Microanalysis," 3rd English ed., P. Blakiston's Son and Co., Philadelphia, Pa., 1937.



Gazaniaxanthin (?)

30% of neo-isomers. Within the same period of time, iodine (1–2% of the pigment) produced an equilibrium mixture containing 45% neo-compounds. Melting of the crystals also produced considerable quantities of stereoisomers.

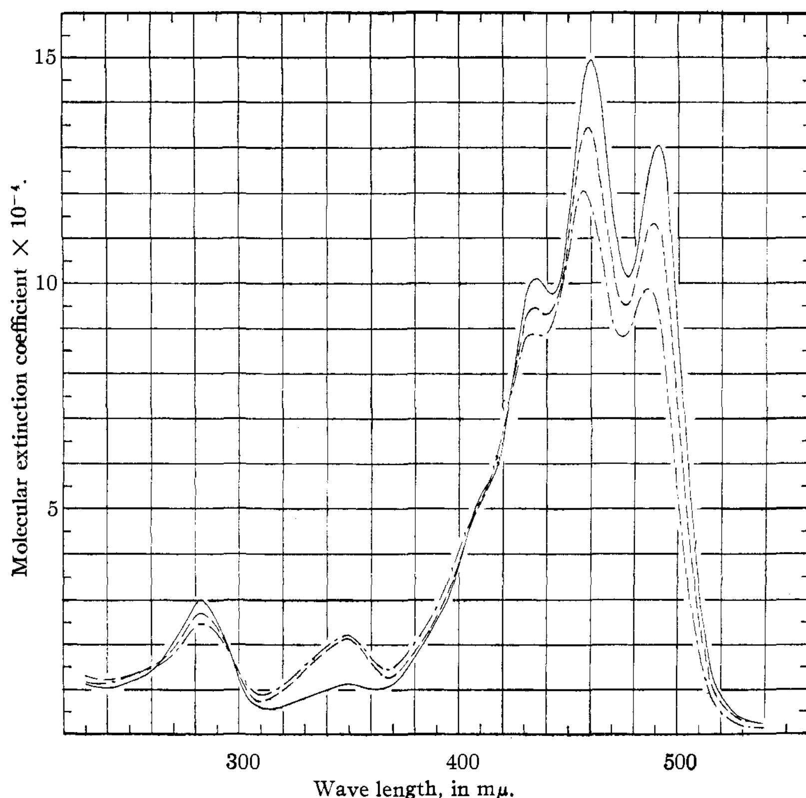


Fig. 1.—Molecular extinction curves of gazaniaxanthin in hexane: ——— fresh solution of the all-*trans* compound; – – – – mixture of stereoisomers after refluxing for forty-five minutes; and —·—· after iodine catalysis for one hour at 25°.

From the standpoint of chromatographic technique, gazaniaxanthin and its isomers represent a difficult case. Even with the best adsorbent and developer available, it was impossible to obtain colorless interzones between the isomers, all of which have less adsorption affinity than gazaniaxanthin. Although the outer appearance of the chromatogram was, at times, excellent, the differentiation inside the column usually did not justify the expectations. However, the stereoisomers of gazaniaxanthin obtained with iodine formed two distinct groups on the column, termed "neo-group I" and "II" (from top to bottom). The average spectra were nearly identical. Within group I, two stereoisomers, within II, four stereoisomers have been differentiated but for none is perfect homogeneity certain. The chromatograms of equilibrium mixtures obtained by refluxing solutions or melting crystals are similar.

As one proceeds down the column in the three cases, the wave lengths of the spectral maxima of the zones first decrease and then increase. The maxima of the middle zones are of 8–10 mμ shorter wave length than those of the all-*trans* compound while this difference is only 3–7 mμ for the bottom zones (see Experimental Section).

The differences in the wave lengths of the absorption maxima of the neogazaniaxanthins are unusually small. The longest wave length maxima (in petroleum ether) of all but one zone were located within the narrow range of 489.5 to 484 mμ. Therefore, the identification of neo-compounds obtained by the different methods of isomerization could not be carried out with fully satisfactory results.

The spectral properties of natural (all-*trans*) gazaniaxanthin have been investigated from 540 to 230 mμ in hexane (Fig. 1). If the solution is treated with iodine at room temperature or merely refluxed, the extinction coefficients decrease and the maxima shift toward the shorter wave lengths. Simultaneously, however, the very flat maximum at 349–350 mμ is greatly increased and converted into a marked maximum. The latter is the so-called "*cis*-peak" defined by Zechmeister and

Polgár.[6] That the equilibria between gazaniaxanthin and its stereoisomers formed by these treatments differ according to the method of isomerization, is evidenced by Fig. 1 as well as by the chromatograms described below.

The *trans-cis* isomerization of gazaniaxanthin induced by iodine catalysis also becomes manifest by the appearance of rather strong dextrorotation. The optical activity of the natural pigment in benzene or petroleum ether was too small to be observed in concentrations which permitted readings. In contrast, the iodine equilibrium mixture gave $[\alpha]^{25}_{Cd}$ +160° (in petroleum ether) and a mixture of some neo-isomers gave $[\alpha]^{25}_{Cd}$ +220° which upon addition of iodine decreased to +155°.

Acknowledgment.—We are indebted to Dr. C. E. P. Jeffreys for carrying out the biological assay of gazaniaxanthin. We also wish to thank Professor A. J. Haagen-Smit as well as Dr. G. Oppenheimer and Mr. G. Swinehart for microanalyses. Mr. W. Hertenstein kindly provided us with the opportunity of collecting the flowers.

Experimental

Materials and Methods.—Calcium hydroxide (Shell Brand lime, chemical hydrate, 98% through 325 mesh) was the adsorbent used unless otherwise indicated. The developers were petroleum ether (b. p. 60–70°) with varying percentages of acetone. Alcohol or ether is suitable as an eluent.

The concentrations of carotenoid solutions were determined with a Pulfrich Gradation Photometer (Zeiss, light filter S 47) and the visual spectra with an Evaluating Grating Spectroscope (Zeiss, light filter BG-7). Spectral data refer to petroleum ether unless otherwise indicated. The readings for the spectral curves were obtained with a Beckman quartz photoelectric spectrophotometer,[7] at intervals of 1 mμ near the maxima and minima and of 5 mμ elsewhere. The values were taken in Eastman Kodak Co. practical hexane which had been purified with sulfuric acid and distilled (b. p. 62–7°). For the rotations a Schmidt and Haensch polarimeter with a cadmium-mercury lamp was used. The possible errors in the angle read were ±0.01–0.02°.

The melting points (cor.) were taken in an electrically heated Berl block in sealed capillaries filled with carbon dioxide.

1. Isolation and Characterization of the Main Carotenoids of *Gazania rigens*

Separation of the Pigment Fractions.[8]—One kilo of petals, collected within a week, was dried at 40–50° for twenty-four hours. The milled material was covered with petroleum ether for two hours in a percolator (35 × 15 × 8 cm.). Percolation was continued until 3.5 liters had passed through. The deep red solution was diluted to 5 liters and saponified with 30% methanolic potassium hydroxide overnight. After cautious addition of water, the petroleum ether phase was washed alkali-free and dried with sodium sulfate. The solution was chromatographed[9] in two percolators (45 × 20 × 8 cm.), development requiring a total volume of 8 liters of petroleum ether and 10 liters of 10% acetone in petroleum ether.

The two chromatograms showed minor top layers followed by a broad red zone of gazaniaxanthin and lycopene which did not separate with this development and were cut out together. Neolycopene and paler zones of cryptoxanthin and γ-carotene were immediately below. β-Carotene and minor pigments were washed into the chromatographic filtrate and discarded. The gazaniaxanthin–lycopene section was eluted with alcohol containing some petroleum ether and the combined cryptoxanthin–γ-carotene zones with alcohol. The carotenoids were transferred into petroleum ether by addition of water, washed alcohol-free and dried.

Isolation of γ-Carotene and Cryptoxanthin.—The petroleum ether solution was developed with this solvent in a percolator (35 × 15 × 8 cm.). The main zones, (*a*) γ-carotene and (*b*) cryptoxanthin, were eluted separately with ether and evaporated to dryness *in vacuo*.

(**a**) **γ-Carotene.**—The residue was dissolved in the minimum amount of cold benzene, transferred into a centrifuge tube and methanol added cautiously with stirring. Upon standing at 5°, a mixture of red and colorless crystals precipitated. After further addition of methanol, the suspension was kept at 5° for another day. It was then centrifuged and the solid repeatedly treated with boiling methanol for a minute or two. Each treatment was followed by rapid centrifuging and decantation of the hot methanol which deposited white crystals upon cooling. These were filtered off. The filtrate, the mother liquor of the first centrifuging and the γ-carotene crystals were combined, rechromatographed and crystallized from benzene–methanol. After a single extraction with hot methanol and recrystallization, the microscope showed homogeneous red crystals of γ-carotene, the form of which was not unlike gazaniaxanthin described below; m. p. 131–133°.[10] The yield of pure γ-carotene was 13.5 mg. In the partition test epiphasic behavior was observed.

The spectral maxima in the visual spectroscope were: in carbon disulfide, 533, 495.5, 462 mμ (with iodine, 530, 492.5, 459 mμ); in benzene, 509.5, 477, 447.5 mμ (506, 473.5 mμ); and in petroleum ether, 495, 462.5, 434 mμ (491.5, 459.5 mμ).

Anal. Calcd. for $C_{40}H_{56}$: C, 89.48; H, 10.52. Found: C, 89.39; H, 10.55.

(**b**) **Cryptoxanthin.**—When the dry residue (see above) was dissolved in a little benzene and diluted with methanol,

(6) L. Zechmeister and A. Polgár, THIS JOURNAL, **65**, 1522 (1943).

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

(8) A quantitative estimation of the individual carotenoids in the flowers may be carried out in a similar manner by using freshly picked petals (corresponding to 2–3 g. of dry weight) which have been dehydrated with methanol.

(9) If chromatography cannot be carried out immediately and the solution must stand in a cold room, it should be filtered before adsorption in order to remove gummy material which will clog the top of the column and may even cause it to split vertically.

(10) For a discussion of the variations in the melting point of γ-carotene, see L. Zechmeister and W. A. Schroeder, *Arch. Biochem.*, **1** 231 (1942).

only colorless material precipitated. After filtering, the pigment content of the filtrate was transferred into ether and saponified with concd. methanolic potassium hydroxide. After washing free of alkali and evaporating to dryness, the residue was chromatographed from petroleum ether, first on calcium carbonate and then on calcium hydroxide. Following elution with ether and evaporation, the dry residue was crystallized from benzene and methanol. The crystals were contaminated with colorless material, and were freed from the latter by recrystallization. The yield was 1.3 mg., m. p. 163°. The behavior in the partition test corresponded to that of cryptoxanthin. In the mixed chromatogram test the sample did not separate from cryptoxanthin obtained from another source. The spectral maxima were: in carbon disulfide, 518, 483.5, 453.5 mμ (with iodine, 513.5, 482 mμ); in benzene, 497, 463.5 mμ (495, 461.5 mμ); and in petroleum ether, 483, 452.5 mμ (481, 450.5 mμ).

Anal. Calcd. for $C_{40}H_{56}O$: C, 86.89; H, 10.22. Found: C, 87.26; H, 10.46.

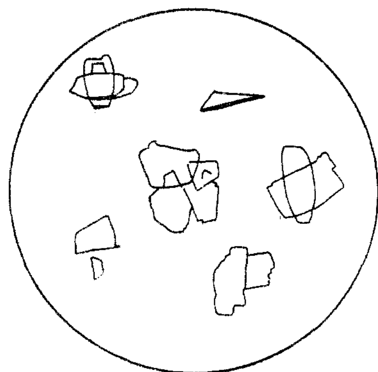


Fig. 2.—Gazaniaxanthin, crystallized from benzene and methanol.

Isolation of Gazaniaxanthin (and Lycopene).—The petroleum ether solution (see above) was rechromatographed in a percolator (35 × 15 × 8 cm.). The hydroxy compound was retained on the column while the lycopene was washed into the filtrate by developing with 5% benzene in petroleum ether. To achieve this separation, Merck calcium carbonate ("Heavy Powder") was employed. The choice of a calcium carbonate which does not retain lycopene is important.

From the filtrate, pure *lycopene* was isolated in a yield corresponding to 135 mg. per kilo of dry petals. The spectral maxima were: in carbon disulfide, 546, 507, 474.5 mμ (with iodine, 543, 502, 469 mμ); in benzene, 521, 486.5, 456 mμ (517.5, 484, 453.5 mμ); and in petroleum ether, 504.5, 473.5, 445 mμ (501, 469.5, 441 mμ).

Anal. Calcd. for $C_{40}H_{56}$: C, 89.48; H, 10.52. Found: C, 89.50; H, 10.71.

The pigment of the main zone, consisting of *gazaniaxanthin*, was eluted with ether and evaporated completely *in vacuo*. The red, crystalline residue was dissolved in the minimum amount of cold benzene, transferred into two 50-ml. centrifuge tubes and crystallized at 25° by gradual addition of excess methanol with stirring. After standing at 5° overnight, the crystals were centrifuged, washed with methanol and dried with a stream of carbon dioxide in the centrifuge tube. Then they were dissolved in benzene at 45°. Methanol was added slowly until the first crystals appeared and then more rapidly. The suspension was kept at 25° for an hour and at 5° for two hours, then filtered. The yield was 560 mg. of pure gazaniaxanthin. After rechromatographing, the mother liquor yielded an additional 65 mg.[11]

The deep rose crystals melted at 133–134°. When suspended before filtration, they had a golden glitter and a rather orange-brown appearance. Under the microscope the crystals were lens-like in form or plates with rounded edges (Fig. 2). The color of individual plates was brownish-orange; superimposed aggregates showed a purplish tint. At times needles were observed grouped in rosets similar to the photomicrographs of rubixanthin.[2]

From petroleum ether solutions, gazaniaxanthin is more strongly adsorbed than lycopene, both on calcium hydroxide and on calcium carbonate. It is adsorbed much above cryptoxanthin.

The partition behavior was identical with that described by Schön.[3]

The spectral maxima in the visible region were: in carbon disulfide, 531, 494.5, 461 mμ (with iodine, 528.5, 491, 458.5 mμ); in benzene, 509, 476, 447.5 mμ (505.5, 473 mμ); in petroleum ether, 494.5, 462.5, 434.5 mμ (491, 459.5 mμ); in hexane, 493.5, 462, 434 mμ (490.5, 459 mμ); and in abs. alcohol, 494.5, 462, 434.5 mμ (no change with iodine). The values of the molecular extinction coefficients for the maxima and minima are given in Table II.

TABLE II

MOLECULAR EXTINCTION COEFFICIENTS OF GAZANIAXANTHIN AND OF ITS STEREOISOMERIC EQUILIBRIA AT THE MAXIMA AND MINIMA IN HEXANE[a]

Fresh solution		After heat isomerization		After iodine isomerization	
mμ	$E^{mol.}_{1cm.} \times 10^{-4}$	mμ	$E^{mol.}_{1cm.} \times 10^{-4}$	mμ	$E^{mol.}_{1cm.} \times 10^{-4}$
491	*13.1*	*489*	*11.3*	*486*	*9.9*
477	10.1	476	9.5	474–5	8.8
460	*15.0*	*458–9*	*13.5*	*456–7*	*12.1*
442	9.8	440	9.3	432–8	8.9[b]
434–5	*10.1*	*434–5*	*9.5*	365	*1.48*
414	5.6[b]	370	1.29	*348–50*	*2.22*
365	1.02	*350*	*2.14*	312	0.8(9)
350	*1.13*	310	0.7(7)	*284*	*2.50*
315	0.5(7)	*282–4*	*2.69*	237	1.11
283	*3.00*	240	1.20		
235	1.03				

[a] The values of the maxima are italicized. [b] Point of inflection.

The concentration of petroleum ether solutions can be determined with the Pulfrich photometer (light filter S 47); k = extinction coefficient, c = mg. of gazaniaxanthin in 100 ml. of solution, and c_I = mg. of iodine equilibrium mixture in 100 ml.

k	0.3	0.5	0.7	0.9
c	0.12(5)	0.21(5)	0.30(5)	0.39(5)
c_I	0.15	0.25	0.36	0.47

(11) In two similar experiments with petals several weeks old, the yields were only 377 and 386 mg. per kilo of dry material.

Anal. Calcd. for $C_{40}H_{54}O$: C, 87.27; H, 9.90. Calcd. for $C_{40}H_{56}O$: C, 86.89; H, 10.22. Calcd. for $C_{40}H_{58}O$: C, 86.58; H, 10.54; mol. wt., 555; double bonds, 11; active hydrogen, 1.0; isopropylidene groups, per mole, 0.0. Found: C, 87.27, 87.41; H, 10.75, 10.54; mol. wt. (in exaltone, $K = 21.3$), 504; double bonds (addn. of hydrogen with PtO_2), 11.2, 11.1, 11.0; active hydrogen (methane evolution), 1.3; isopropylidene groups per mole (acetone by $N/20$ iodine, corrected for blank[12]), 0.85, 0.85.

Polarimetry.—With concentrations between 0.05 and 0.1 g. per 100 ml. using 1-dm. tubes, no rotations were observed in benzene or petroleum ether, while chloroform solutions showed a moderate rotation: $[\alpha]^{25}_{Cd} = +(100 \times 0.06°):(1 \times 0.092) = +65°$ (in $CHCl_3$). Upon the addition of a drop of concentrated iodine solution to the polarimeter tube, the rotatory power increased within a few minutes: $[\alpha]^{25}_{Cd} = +(100 \times 0.14°):(1 \times 0.092) = +150°$ (in $CHCl_3$). On the other hand, if a petroleum ether solution of gazaniaxanthin was catalyzed with iodine and chromatographed, the heterogeneous neo-section located immediately below the zone of unchanged gazaniaxanthin gave: $[\alpha]^{25}_{Cd} = +(100 \times 0.10°):(1 \times 0.046) = +220°$ (in petroleum ether). A drop of iodine produced a decrease in the rotation: $[\alpha]^{25}_{Cd} = +(100 \times 0.07°):(1 \times 0.046) = +155°$ (in petroleum ether).

2. *Cis–trans* Isomerization of Gazaniaxanthin

For the separation of gazaniaxanthin and its neo-forms the choice of the adsorbent and developer is of more than usual importance because the zones tend to yield an undifferentiated sequence on the column. The following adsorbents gave no separation: alumina (used by Schön with success in the isolation of gazaniaxanthin), zinc carbonate, calcium carbonate, calcium carbonate–hydroxide mixtures, magnesium carbonate, basic magnesium carbonate, and magnesium oxide. It was only on the calcium hydroxide mentioned above that a separation of gazaniaxanthin from its stereoisomers and a further differentiation of the latter took place after prolonged development with petroleum ether containing at first 5, later 10 and finally 25% acetone. This procedure was used in all experiments described below. In the range of 5–25 mg. such a development required one to three hours and was usually continued until the lowest pigment zone neared the bottom of the column. When the isomerization mixture obtained from 25 mg. of gazaniaxanthin was chromatographed on a 28 × 7 cm. column, 2–3 liters of the developer was required of which 4/5 was 25% acetone in petroleum ether.

(a) **Isomerization of Gazaniaxanthin by Iodine Catalysis at Room Temperature.**—A solution of 25 mg. of pigment in 3 ml. of cold benzene was diluted to 100 ml. with petroleum ether and treated with a solution containing 0.5 mg. of iodine. After standing an hour at 25° in diffuse light, the mixture was developed on a column (28 × 7 cm.) as described above.

110 colorless top section
60 pink, gazaniaxanthin: 494.5, 462.5, 434.5 mμ (with iodine, 490.5, 458.5 mμ)
18 brownish orange: 487.5, 457 mμ (491, 459 mμ)
15 lighter brownish orange: 487, 457 mμ (491, 459 mμ)
1–2 nearly colorless (between Groups I and II)
8 yellow: 484, 454 mμ (491, 459 mμ)
14 orange yellow: 485, 454.5 mμ (491, 459 mμ)
9 orange: 486, 456 mμ (491, 459 mμ)
5 pink: 487.5, 456.5 mμ (491, 459 mμ)

In other experiments portions of the catalyzed solution were adsorbed on columns after five, fifteen, and thirty minutes. Following elution with alcohol and transference into petroleum ether these colorimetric ratios were found

Catalysis min.	0	5	15	30
Gazaniaxanthin:				
Group I:				
Group II.	100:0:0	72:20:8	56:29:15	56:29:15

With neo-compounds as a starting material the equilibrium was also attained within fifteen minutes.

The corresponding weight ratio at equilibrium was 50:30:20. Such figures can be obtained by the addition of iodine to each of the chromatographically separated pigment zones and the use of photometric values ("c_I") for the iodine equilibrium mixture given in Section 1.

(b) **Isomerization by Refluxing.**—For the best possible separation of isomers formed by this method it is essential that the ratio of adsorbent: pigment be 2 to 3 times that used in experiments with iodine (see above). Fifteen mg. of gazaniaxanthin in 100 ml. of benzene was refluxed for an hour. After cooling rapidly to room temperature and diluting with 1 vol. of petroleum ether, a chromatogram was obtained (28 × 7 cm.) from which all isomers and a small section of the unchanged gazaniaxanthin were cut out together. After elution with alcohol this mixture was rechromatographed (25 × 5 cm.).

90 colorless top section
50 pink: gazaniaxanthin, 494.5, 462.5, 434.5 mμ
3 brownish pink: 488.5, 457.5 mμ (with iodine, 491, 459 mμ)
15 brownish pink: 487.5, 456.5 mμ (490.5, 458.5 mμ)
20 brownish orange: 486.5, 456 mμ (490.5, 458.5 mμ)
20 orange: 486, 455.5 mμ (490.5, 459 mμ)
25 orange brown: 487.5, 456.5 mμ (490, 459 mμ)
7 pink: 489.5, 458.5 mμ (491, 459.5 mμ)

The approximate rate of isomerization was established in a separate experiment

Refluxing, min.	0	15	30	60
Gazaniaxanthin: neo-forms	100:0	71:29	70:30	68:32

(c) **Isomerization of Solutions at Room Temperature.**—Ten mg. of gazaniaxanthin in 25 ml. of petroleum ether was kept in the dark under carbon dioxide at 25–28°. Five-ml. portions were chromatographed periodically (column, 20 × 1.9 cm.). After cutting unchanged gazaniaxanthin from all its isomers, the relative color intensities of the two eluates were estimated in petroleum ether

Time, days	0	1	2	4	8
Gazaniaxanthin: neo-forms	100:0	96:4	93:7	88:12	82:18

Neogazaniaxanthins are relatively stable under these conditions. For example, starting with a (chromatographically heterogeneous) neo-section which was located immediately below the all-*trans* zone, about two days were required to reach the equilibrium.

Time, days	0	1	2	4
Gazaniaxanthin: neo-forms	0:100	38:62	46:54	45:55

(12) For simultaneous control determinations, see note (10).

(d) **Isomerization by Melting.**—A sealed tube containing 20 mg. of the carotenoid was maintained at 140–145° for five minutes. After plunging into ice water, the solidified mass was taken up in cold benzene and chromatographed (28 × 7 cm.), following dilution with petroleum ether.

100 colorless top section
60 pink: gazaniaxanthin: 494.5, 462.5, 434.5 mμ (with iodine, 490.5, 458.5 mμ)
5 brownish pink: 489, 457 mμ (490.5, 459 mμ)
20 orange brown: 488, 456.5 mμ (491, 458.5 mμ)
15 orange brown: 485, 454.5 mμ (490.5, 458.5 mμ)
15 yellow: 484, 453.5 mμ (491.5, 459 mμ)
15 yellow: 486, 454.5 mμ (491, 458.5 mμ)
12 pink: 489.5, 457.5 mμ (492, 459 mμ)
1 traces of color: 491, 460.5 mμ (491, 459 mμ)

Summary

1. Gazaniaxanthin,[3] the main pigment of *Gazania rigens* flowers, has been studied by means of methods causing *trans–cis* changes in solution and in melt. On the Tswett column two groups of neo-compounds appeared, the further differentiation of which was difficult.

2. The molecular extinction curve of all-*trans*-gazaniaxanthin shows a very flat maximum around 350 mμ. Upon heating or iodine catalysis, however, a marked maximum develops in this region ("*cis*-peak").[6]

3. Catalytic hydrogenation indicates that gazaniaxanthin may be dihydrorubixanthin.

4. The carotenoid mixture in *Gazania* flowers grown in Portugal[3] differs from that occurring in flowers grown in Southern California.

PASADENA, CALIFORNIA RECEIVED MARCH 4, 1943

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

The Effect of Oxygen on the Fluorescence of Certain Hydrocarbons[1]

BY J. A. MILLER AND C. A. BAUMANN

Oxygen is known to diminish the fluorescence of many compounds[2,3,4,5] including certain polycyclic hydrocarbons.[2,3,4] Weil-Malherbe and Weiss[2] observed that the fluorescence of benzpyrene was nearly the same in several solvents from which oxygen had been removed by suction, but that on oxygenation varying degrees of quenching resulted. The quenching effect was greater in oxygen than in air, and was completely and rapidly reversible. Recently we have observed that the fluorescence of several carcinogenic hydrocarbons varies markedly with the solvent employed.[6] The results did not, however, indicate to what extent the variations were due to dissolved oxygen or directly to the solvent itself.

Accordingly the effect of oxygen has been measured on the fluorescence of 3,4-benzpyrene (BP), 20-methylcholanthrene (MC), 9,10-dimethyl-1,2-benzanthracene (DMBA), 1,2,5,6-dibenzanthracene (DBA), 1,2-benzanthracene (BA), and anthracene (A), each in several representative solvents. Low concentrations of hydrocarbon were employed. The solutions were placed in long all-glass fluorometer tubes as previously described,[7] and the dissolved air removed at the pump. The intensity of fluorescence was measured in a Coleman photofluorometer[7] *in vacuo*[8] and again after shaking the solution with known mixtures of oxygen and nitrogen. Deductions were then made regarding the composition of a hydrocarbon–inhibitor complex assumed to be present in the quenched solutions. Other gases and other inhibitors of fluorescence were studied in a similar manner, and in certain cases the effect of the inhibitor on the hydrocarbon compared with its effect on the unsaponifiable matter of mouse tissues.

General Results

In vacuo, the fluorescence of dilute solutions of benzpyrene was nearly the same in the six solvents employed (Table I). In air, however, marked variations with solvent were observed, and usually the readings were less than half of those observed *in vacuo*. This parallels the results of Weil-Malherbe and Weiss.[2] However, in tetrahydrofurfuryl alcohol, the fluorescence observed in air was over 85% of that observed *in vacuo*, while in aniline or in carbon tetrachloride

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(2) Weil-Malherbe and Weiss, *Nature*, **149**, 471 (1942).
(3) Bowen and Norton, *Trans. Faraday Soc.*, **35**, 44 (1939).
(4) Bowen and Williams, *ibid.*, **35**, 765 (1939).
(5) Kautsky, *Ber.*, **64**, 2677 (1931).
(6) Miller and Baumann, *Cancer Research*, **3**, 223 (1943).
(7) Miller and Baumann, *ibid.*, **3**, 217 (1943).
(8) In this paper the phrase "*in vacuo*" refers to the absence of all gas but solvent vapor.