THE FRUIT OF PYRACANTHA ANGUSTIFOLIA: A PRACTICAL SOURCE OF PRO-γ-CAROTENE AND PROLYCOPENE

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While the generally known and wide-spread C_{40} carotenoids presumably possess an all-*trans* configuration, two representatives of a stereochemically new class of naturally occurring polyenes were recently described (1, 2). Of the seven and six double bonds sterically available for *trans-cis* shifts, prolycopene and pro-γ-carotene contain 5–6 and 4–5 *cis* double bonds respectively. On addition of iodine to solutions of these compounds, the corresponding all-*trans* isomer is formed in each case together with minor stereoisomers. This becomes evident by an instantaneous shift in the spectra. After catalysis, the first maxima are located at 34 and 32 mμ longer wave-length respectively than those of the original solutions.[1]

Prolycopene, $C_{40}H_{56}$, was first isolated from the tangerine tomato (a variety of *Lycopersicum esculentum*) in a yield of 20.6 mg. per kilo of fresh material (3). Much less satisfactory was the yield of pro-γ-carotene, $C_{40}H_{56}$; *viz.*, 0.3 mg. per kilo of fresh palm fruit (*Butia capitata* Becc.) (4). In the interests of further stereochemical work, a great variety of plant material was tested for "pro" carotenoids in our laboratory by means of extraction, chromatography, and iodine catalysis.

As a result of these analyses, it has been found that the ripe fruit of *Pyracantha* (*Cotoneaster*) *angustifolia* Schneid. (Pomoideae) constitutes the only practical source of pro-γ-carotene at the present time; 27.7 mg. were obtained in crystalline form from 1 kilo of air-dried berries (about 3 kilos of fresh material). The same quantity also yielded 28.4 mg. of prolycopene. Furthermore, a second member of the stereoisomeric series, lycopene-prolycopene, was isolated (7.3 mg. of crystals) but we cannot claim with certainty that it is a natural product.

Since all the pigments mentioned are hydrocarbons, the question arises whether the occurrence of "pro" compounds in the vegetable kingdom is restricted to this type. A minor constituent of the *Pyracantha* pigment gave information on this point. Since its spectrum is identical with that of pro-γ-carotene before and after the addition of iodine, both must

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[1] All spectral data refer to solutions in petroleum ether (b.p. 60–70°) unless otherwise indicated.

possess a similar chromophore. On the other hand, the behavior of the compound in the partition test and especially its increased adsorption affinity as compared with pro-γ-carotene (and even γ-carotene) prove the presence of a hydroxyl group. Because of the small quantities available, this monohydroxy pro-γ-carotene has not yet been prepared in crystalline form.

It should be noted that all "pro" compounds known at the present time possess at least one aliphatic end-group in their molecules.

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EXPERIMENTAL

The *Pyracantha* berries were picked in November and December in Southern California and dried in air at room temperature. On prolonged standing the yields diminish rapidly. 1 kilo of the air-dried material was coarsely ground in a mill, kept under peroxide-free ether for 3 hours, then filtered on a Buchner funnel, washed with ether, and treated once more in the same manner. The extract (4.5 liters) was saponified for a day by keeping it over concentrated methanolic potassium hydroxide. After addition of water the ether solution was washed alkali-free. (The dark wash water did not contain carotenoids.) The solution was dried rapidly with sodium sulfate and evaporated. In order to remove small quantities of ether which would disturb the subsequent adsorption, some petroleum ether was added and the evaporation repeated.

The solution of the dark red, viscous residue in 1 liter of petroleum ether was chromatographed in a large percolator (45 × 20 × 8 cm.) on calcium hydroxide (Shell brand lime, chemical hydrate, 98 per cent passing through a 325 mesh screen). The chromatogram was developed with 5 liters of petroleum ether and then with the same solvent containing 1 per cent acetone. The complicated chromatogram was composed of three sections: (*a*) a strongly adsorbed, poorly differentiated top section (7 cm. wide), (*b*) a main section (6 cm.) containing several orange and yellow zones including prolycopene and pro-γ-carotene, and (*c*) the lowest section of the cone, occupied by large amounts of β-carotene preceded and followed by some of its stereoisomers. This last section and the yellow, fluorescing filtrate were discarded.

The percolator was inverted and the cone removed in one piece by tapping on the glass with the palm of the hand; the three sections were separated by cutting.

Fractionation of Main Section—After elution with alcohol the pigments

were transferred to petroleum ether and developed on a calcium hydroxide column (28 × 7 cm.) with petroleum ether containing 2 per cent acetone. The chromatogram had the following appearance (the figures on the left denote the width of the zones).

80 mm., several minor layers near top
35 " bright orange, prolycopene (470.5, 441 mμ)
5 " orange, traces
12 " yellow, unidentified Pigment 1 (464, 438 mμ)
10 " several minor layers
50 " orange, pro-γ-carotene (462, 432.5 mμ)
10 " yellow, unidentified Pigment 2 (457.5, 430.5 mμ)
20 " several minor layers

The presence of pro-γ-carotene and of prolycopene was detected by the addition of iodine to the respective solutions in a spectroscopic cell. By this catalysis intense new spectra appeared almost instantaneously (493, 460 mμ and 501.5, 470 mμ).

Pro-γ-carotene—This zone was rechromatographed and developed with petroleum ether containing 2 per cent acetone; the main component was eluted with ether. Upon evaporation of the ether *in vacuo* a dark red, crystalline residue remained. The latter was dissolved in the minimum amount of benzene at 20° and transferred into a 15 cc. centrifuge tube with a dropper. About 10 cc. of methanol were then added with stirring, first drop by drop until red crystals appeared, and later more rapidly. After standing in ice water for ½ hour, the crystals were centrifuged and washed with methanol in the same tube. After recrystallization from benzene and methanol, the yield was 25.1 mg. The mother liquor gave 2.6 mg. The total yield corresponds to 45 per cent of the pro-γ-carotene content of *Pyracantha* as estimated photometrically. M.p., 121–122° (corrected) (after softening near 119°; the sealed tube was filled with CO_2; the sample was introduced into the Berl block 20° below the melting point). The crystal form has been described (4). The partition behavior (petroleum ether-95 per cent methanol) was epiphasic. A mixed chromatogram with pro-γ-carotene from *Butia capitata* established the identity of the two samples. For the purpose of analysis the substance was dried at 50° in a high vacuum for 45 minutes. It was free of ash.

Analysis—$C_{40}H_{56}$. Calculated. C 89.48, H 10.52
Found. " 89.79, 89.94, " 10.55, 10.52
Mol. wt. (in exaltone), calculated, 537; found, 558

The absorption maxima in carbon disulfide were 492.5, 459 mμ (after the addition of iodine, 529.5, 491.5 mμ); in benzene, 475.5, 446.5 mμ (with iodine, 506.5, 472 mμ); and in petroleum ether, 462, 432.5 mμ, (with iodine,

493, 460 mμ). In all cases a slight shadow was observed at about 30 mμ longer wave-length than the first maximum.

Prolycopene—This layer was cut out and rechromatographed. By developing with petroleum ether containing 5 per cent acetone, minor layers were separated. The main pigment was then eluted and isolated as described for pro-γ-carotene. A recrystallization was not carried out in this case. The yield was 28.4 mg.; *i.e.*, about 40 per cent of the quantity contained in the original extract as estimated photometrically. M.p., 110.5–111.5° (corrected). The shape of the crystals and their solubility corresponded with those of a sample from tangerine tomatoes. A mixed chromatogram showed no separation. In the partition test (petroleum ether-95 per cent methanol) epiphasic behavior was observed. For the purpose of analysis the sample was dried in a high vacuum at room temperature for 45 minutes.

Analysis—$C_{40}H_{56}$

Calculated. C 89.48, H 10.52
Found. " 89.39, " 10.63 (corrected for 0.7 % ash)
Mol. wt. (in exaltone), calculated, 537; found, 575

The absorption maxima in carbon disulfide were 500, 468 mμ (with iodine, 542.5, 502.5, 468.5 mμ); in benzene, 482.5, 453 mμ (with iodine, 518.5, 483, 452.5 mμ); and in petroleum ether, 470.5, 441 mμ (with iodine, 501.5, 469.5, 439.5 mμ).

Minor Pigments—The unidentified Pigment 1, after having been rechromatographed, showed absorption maxima at 464.5, 435.5 mμ which were shifted on iodine catalysis to 469, 439 mμ. This equilibrium mixture when chromatographed and developed with petroleum ether containing 5 per cent acetone separated into five layers; the spectrum of the main pigment, adsorbed near the top, was 471, 442 mμ.

The unidentified Pigment 2, after having been rechromatographed and treated with iodine, separated upon chromatographing into two isomers (458, 431 mμ and 454, 426 mμ respectively, from the top of the column) which gave identical spectra (457, 429 mμ) on treatment with iodine.

Fractionation of Top Section—The pigments were eluted with alcohol, transferred to petroleum ether, and developed on calcium hydroxide in a smaller percolator (30 × 11 × 6 cm.) with the solvent mentioned containing 5 per cent acetone. Five main fractions (Fractions I to V from top to bottom) appeared, each consisting of several pigments.

Fraction I (which among other pigments contained some lutein) and Fraction III were of no particular interest.

Fraction II, when rechromatographed and developed with 10 per cent acetone in petroleum ether, separated into nine components; *viz.*, lycopene, two neolycopenes, a monohydroxy pro-γ-carotene, and five minor layers.

The monohydroxy pro-γ-carotene zone was rechromatographed and was then homogeneous. The main absorption maxima were 461.5, 432.5 mμ and on iodine catalysis, 492.5, 459 mμ. In contrast to pro-γ-carotene this pigment was present in both phases if a drop of water was added to the solution in a petroleum ether-methanol mixture. When a petroleum ether solution was shaken with 90 per cent methanol, epiphasic behavior was observed. The adsorbability is also conclusive evidence for the presence of a hydroxyl group. On calcium hydroxide the compound is adsorbed below lycopene but much above pro-γ-carotene, as shown by mixed chromatograms. Furthermore, after the addition of iodine a main component is formed which does not separate on the column from a monohydroxy-γ-carotene (probably rubixanthin) obtained from another source. Finally, monohydroxy pro-γ-carotene is also adsorbed on calcium carbonate from petroleum ether, which, as is well known, does not occur with hydrocarbon carotenoids.

Fraction IV consisted mainly of an orange pigment which showed maxima at 474, 442.5 mμ (with iodine, 501.5, 469.5, 439 mμ). In carbon disulfide the corresponding figures were 504, 471.5 mμ and 543, 502.5, 468 mμ. This stereoisomer of lycopene was eluted with ether and crystallized as described above for pro-γ-carotene. The yield was 7.3 mg.; m.p., 97–98° (corrected) (after softening). The main pigment formed by iodine catalysis did not separate from tomato lycopene in a mixed chromatogram.

Analysis—$C_{40}H_{56}$. Calculated. Mol. wt. 537
Found. " " 563 (in exaltone)

Fraction V, when rechromatographed on calcium hydroxide with 25 per cent ligroin in benzene, separated into several minor carotenoids and two main pigments with γ-carotene spectra (495, 462 mμ and 494, 461 mμ). On addition of iodine maxima of shorter wave-length appeared. Both have been crystallized and will be investigated. As remarked earlier (4) some observations seem to point to the existence of two γ-carotenes, which possibly differ only in the position of the isolated double bond.

SUMMARY

Ripe fruit of *Pyracantha* (*Cotoneaster*) *angustifolia* Schneid. constitutes the best practical source for the isolation of pro-γ-carotene, $C_{40}H_{56}$, and a good source for prolycopene, $C_{40}H_{56}$, both of which possess partially *cis* configurations. The yields were 27.7 mg. of crystallized pro-γ-carotene and 28.4 mg. of prolycopene from 1 kilo of air-dried berries. A close stereoisomer of prolycopene was also isolated (7.3 mg.) and a monohydroxy pro-γ-carotene observed in solution.

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