ISOLATION OF PROLYCOPENE AND PRO-γ-CAROTENE FROM EVONYMUS FORTUNEI

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It has been reported (1) that the ripe seeds of *Evonymus europaeus* L. (Celastraceae) contain unesterified zeaxanthin, $C_{40}H_{56}O_2$, as the main polyene pigment. The same statement is valid for *Evonymus fortunei*, var. color., Rehd., commonly termed "winter-creeper euonymus," from 1 kilo of which 1300 mg. of zeaxanthin were isolated. Upon evaporation of its saponified ether extract and addition of petroleum ether, abundant quantities of zeaxanthin crystallize. This paper describes an investigation of some of the pigments (about 200 mg. per kilo, of which one-fourth is β-carotene) which remain in the mother liquor. The mixture can be resolved by chromatographic analysis. In addition to some twenty less interesting pigments, two representatives of a stereochemically new class of natural carotenoids (2) were separated; *viz.*, prolycopene, $C_{40}H_{56}$, and pro-γ-carotene, $C_{40}H_{56}$. The yields of pure crystals were 11 mg. and 0.5 mg. per kilo of seeds respectively. Hence, *Evonymus fortunei* may serve as a source of prolycopene while it does not offer any larger yield of pro-γ-carotene than does the fruit of *Butia capitata* (3). According to the foregoing paper (4) *Pyracantha angustifolia* is the best starting material for the isolation of pro-γ-carotene at the present time.

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EXPERIMENTAL

The material was collected in Denton, Texas. The intensely orange-red hulls of 1 kilo of seeds were scraped off by rubbing between two layers of wire gauze in a mortar. Small particles of hull remained on the stones and were neglected. The pigment- and lipid-rich hulls were ground with sand and extracted with peroxide-free ether by repeated shaking. The dark extract (2 liters) was saponified over concentrated methanolic potassium hydroxide for 20 hours, then washed alkali-free, dried with sodium sulfate, and evaporated *in vacuo* at 40° as far as possible. To the dark, partially

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crystalline residue, petroleum ether (b.p. 60–70°) was repeatedly added and evaporated. Finally, the oily residue was dissolved in the minimum volume of chloroform. On addition of 3 volumes of petroleum ether the main bulk of zeaxanthin crystallized out.

The mother liquor was poured on calcium hydroxide (Shell brand lime, chemical hydrate, 98 per cent passing through a 325 mesh screen) in a percolator (45 × 20 × 8 cm.). After washing the chloroform from the adsorbent with petroleum ether, the chromatogram was developed in the course of 3 hours with petroleum ether containing 2 per cent and later 3 per cent acetone. The light orange filtrate was discarded; it contained among other polyenes a portion of the β-carotene.

The cone was cut into three parts. The upper section (160 mm. from the top) was composed of an orange-brown (20 mm.) and a red (140 mm.) part, both of which were heterogeneous. Then followed an orange section (100 mm.) containing the two "pro" compounds and some minor pigments. In the lowest section β-carotene predominated (60 mg.). The middle section was eluted with alcohol, transferred to petroleum ether, and developed on a calcium hydroxide column (28 × 7 cm.) with 1 liter of petroleum ether containing 2.5 per cent acetone and then with 0.5 liter containing 4 per cent. The following chromatogram appeared (on the left side the height of the zones is given).

55	mm.,	pink
40	"	orange, contained prolycopene
4	"	greenish yellow
3	"	light orange
5	"	yellow
20	"	orange, contained pro-γ-carotene
7	"	greenish yellow
15	"	faint orange
0.5	"	green line
15	"	colorless
7	"	pink, β-carotene

Prolycopene—This zone was cut out, eluted with ether, dried, and evaporated *in vacuo*. The residue was dissolved in the minimum amount of benzene and crystallized in a centrifuge tube by cautious addition of several volumes of methanol. The microscope showed typical prolycopene crystals intermixed with much colorless crystalline material. The latter could not be removed by recrystallization from benzene and methanol and only partially by treatment with methanol at 40°. It was almost completely removed by short centrifuging at slow speed. The heavy pigment crystals settled and the suspended colorless compound was decanted. Minor amounts of prolycopene in the decanted liquid were recovered by repeating the process. The last trace of the contaminant was removed

by a short treatment with methanol at 40° and rapid centrifuging. The yield was 11 mg.; m.p., 109–110° (corrected; in a sealed tube filled with CO_2). For the purpose of analysis the sample was dried at about 45° 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. " 89.00, " 10.72
Mol. wt., calculated, 537; found, 529 (in exaltone)

In a mixed chromatogram the pigment did not separate from prolycopene obtained from tangerine tomatoes (2). The spectral maxima of fresh solutions were in carbon disulfide 500.5, 469.5 mμ (after the addition of iodine, 540.5, 500.5, 466 mμ) and in petroleum ether 470, 442 mμ (with iodine, 501, 469, 441 mμ).

Pro-γ-carotene—This zone of the above chromatogram was eluted with alcohol, transferred into 20 cc. of petroleum ether, and rechromatographed on a calcium hydroxide column (27 × 5 cm.). Minor layers located both above and below the main orange pigment were discarded and the latter was rechromatographed on a smaller column (20 × 3 cm.) of the same adsorbent. This showed only traces of other pigments, much above pro-γ-carotene. The latter was eluted with ether and the evaporation residue was crystallized from benzene and methanol as described for prolycopene. No colorless contaminant was present. The yield was 0.5 mg.; the crystal form was typical for pro-γ-carotene. In a mixed chromatogram no separation took place from a sample isolated from *Pyracantha angustifolia* (4). Spectral maxima in carbon disulfide were 492.5, 459 mμ (with iodine, 527.5, 490 mμ) and in petroleum ether, 461.5, 431.5 mμ (with iodine, 490, 457.5 mμ).

SUMMARY

From 1 kilo of the ripe seeds of *Evonymus fortunei* Rehd., 11 mg. of prolycopene, $C_{40}H_{56}$, and 0.5 mg. of pro-γ-carotene, $C_{40}H_{56}$, have been obtained in crystalline form.

BIBLIOGRAPHY

1. Zechmeister, L., and Szilárd, K., *Z. physiol. Chem.*, **190,** 67 (1930). Zechmeister, L., and Tuzson, P., *Z. physiol. Chem.*, **196,** 199 (1931).
2. Zechmeister, L., LeRosen, A. L., Went, F. W., and Pauling, L., *Proc. Nat. Acad. Sc.*, **27,** 468 (1941). LeRosen, A. L., and Zechmeister, L., *J. Am. Chem. Soc.*, **64,** 1075 (1942). Zechmeister, L., and Schroeder, W. A., *Science*, **94,** 609 (1941).
3. Zechmeister, L., and Schroeder, W. A., *J. Am. Chem. Soc.*, **64,** 1173 (1942).
4. Zechmeister, L., and Schroeder, W. A., *J. Biol. Chem.*, **144,** 315 (1942).