SILK OAK FLOWERS AS A SOURCE OF β-CAROTENE

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The pigment of the yellow flowers of the silk oak (*Grevillea robusta*, Cunningham) does not appear to have been investigated heretofore. If the dried material is extracted with ether, the solution shows typical absorption maxima at 483 and 453 mμ, corresponding to the spectrum of β-carotene. The rather blurred borders of these bands indicate, however, the presence of other polyenic pigments in small quantities. After saponification a photometric analysis of the total extract gave values which would correspond to 270 mg. of β-carotene in 1 kilo of the dry flowers if no other pigments were present. After a chromatographic separation the true β-carotene content was found to be about 215 mg. per kilo. Two-thirds of this amount was isolated as crystals; lycopene or γ- and α-carotene were not present.[1] The non-carotene fraction is a complicated xanthophyll mixture in which no single compound predominates. From this fraction two very small amounts of crystalline material were isolated, one of which was kryptoxanthin and the other a new carotenoid possessing a remarkably short wave-length spectrum.

For the separation and study of carotenoids contained in extracts we suggest the systematic use of the ultraviolet lamp which has been so helpful in the chromatography of colorless substances (2). Plant pigments are frequently accompanied by large amounts of colorless material which prevent the formation of sharp pigment zones in the Tswett column and thus a satisfactory separation of the components. Furthermore, the crystallization of some carotenoids may be hindered. Fortunately many such colorless sub-

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[1] In the leaves β- but not α-carotene was found by Strain (1).

stances show an intense fluorescence (3). An observation made in ultraviolet light during the chromatographic separation of the pigments may furnish a good indication of the best method and optimum extent of developing the chromatogram. The distribution of the fluorescence may also indicate the lines at which it is best to cut the column. By sacrificing small amounts of pigment large portions of colorless associated material may be eliminated in this simple way.

EXPERIMENTAL

The flowers were dried in air and then on sieves, over electric bulbs, at 40–45°. The milled material (17.7 kilos) was percolated with ether and the extract was saponified overnight with concentrated methyl alcoholic potassium hydroxide. The soaps and the alkali were carefully washed out; the ether solution was dried with sodium sulfate and evaporated. The dark oil was dissolved in 1.5 liters of petroleum ether (b.p. 60–70°) and chromatographed on calcium hydroxide (Shell). For this purpose it is convenient to use two percolators (20 × 50 × 8 cm.). The chromatogram was developed with petroleum ether until the main bulk of the β-carotene formed a dark orange layer, located two-thirds of the way down the column. It was rather well, if not sharply, separated by a pale intermediate zone from the other pigments which formed a blurred section above the β-carotene. The filtrate showed an intense bluish fluorescence. Such a conic adsorption "column" cannot be pressed out but when the reversed percolator is gently tapped the whole cake comes out in one piece.

The β-carotene layer was cut out, eluted with ethyl alcohol, and rechromatographed from petroleum ether as described, this time in cylindrical tubes (30 × 8 cm.). Some small accompanying layers were discarded. The β-carotene section was eluted and transferred into petroleum ether from which it crystallized on evaporation. The crude product was dissolved in hot petroleum ether and absolute ethanol was added. The latter procedure yielded 2.6 gm. of well crystallized, optically inactive β-carotene. Maxima, in carbon disulfide 520, 486 mμ and in petroleum ether 484.5, 454 mμ. After further recrystallization from benzene and methanol the melting point was 179.5° (Berl block, short thermometer, uncorrected).

Analysis—$C_{40}H_{56}$. Calculated. C 89.48, H 10.52
Found. " 89.43, 89.30, H 10.60, 10.50

On repeated chromatography of the carotenoids which were adsorbed above the β-carotene, at least twenty narrower pigment layers were observed, some of which had been formed by isomerization. Several strongly fluorescing sections were eliminated. Small amounts of lutein and kryptoxanthin were identified spectroscopically and by mixed chromatography. A few mg. of the former were obtained in crystals. One of the other layers contained a new carotenoid showing the following maxima: in carbon disulfide 490.5, 457 mμ; in benzene 479.5, 440.5 mμ; in petroleum ether 457.5, 430 mμ. This pigment crystallized in long plates. The yield was less than 1 mg.

SUMMARY

0.15 gm. per kilo of crystallized β-carotene has been isolated from dried silk oak flowers (*Grevillea robusta*, Cunningham). About one-fifth of the total pigment is a complicated xanthophyll mixture.

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