The Preparation of β -carotene of a High Degree of Purity

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The preparation of 20 g. of β -carotene of a high degree of purity was undertaken in these laboratories at the request of the Medical Research Council.

This relatively large sample, which was prepared from a carotene concentrate obtained from alfalfa meal, had physical characteristics which indicated a purity similar to that of the β -carotene previously isolated from 'Sherbro' palm oil (Hunter & Scott, 1941). The ultra-violet absorption spectrum of a

the bulk of the carotene which can be isolated. The crude carotene was washed in turn with ethanol, water, and again ethanol and dried in a vacuum desiccator.

The mother liquor was twice shaken with water and kept overnight, when part of the contaminating wax crystallized. The upper layer was syphoned off and the residual mixture was thrice extracted with benzene-light petroleum in a 10 l. bottle. Separation of the layers was best effected by warming the bottle in a water-bath. The combined extracts were washed until neutral, concentrated under reduced CO_2

Fig. 1. Absorption curve of β -carotene in cyclohexane.

fresh solution in cyclohexane showed no inflexion at 330–340 mμ (Fig. 1), confirming the absence of *cis*-isomers (Zechmeister & Polgar, 1943).

A smaller sample of this carotene was then subjected to five further recrystallizations. The spectroscopic values of the specimen thus obtained showed little change but were appreciably higher than those recorded in the recent literature.

EXPERIMENTAL

The carotene concentrate (obtained from the Chlorophyll Corporation, Inc., U.S.A.; the containing medium was cotton-seed oil) showed an $E_{1\%1\text{cm}}$ value of 127 at 460 mμ in chloroform. It was saponified and worked up in two batches as follows. The concentrate (1 kg.) in warm light petroleum (2 l.) was heated, under reflux, with NaOH (500 g.) in water (500 ml.) and 95% ethanol (3 l.) for 3 hr. Warm light petroleum (3 l.) was added, and after $\frac{1}{2}$ hr. the supernatant liquid was syphoned off from the residue, which constitutes

pressure and cooled to 0°, when wax again separated. Further quantities of wax and sterol were removed by crystallization from acetone and finally light petroleum.

The concentrated mother liquor was chromatographed, under slight CO_2 pressure (Hunter & Scott, 1941), on columns of activated alumina (70 × 4.2 cm. diam.). Development of the columns with 1 l. light petroleum sufficed to wash out the relatively small orange-red zone of carotene. The combined percolates were evaporated with the usual precautions and the residue crystallized at 0° from benzene-acetone. This product (8 g.) was added to the main crude carotene (obtained initially from the saponification mixture) and the whole treated four times with boiling light petroleum and twice with boiling ethanol to remove as much wax and sterol as possible. This yielded 70 g. of crude carotene from 2 kg. of concentrate.

Chromatography. Batches of 2.5 g. of the carotene in pure benzene and light petroleum (1:1, 370 ml.) were chromatographed on columns of activated alumina (900 g., 75 × 4.4 cm. diam.) under CO_2 , and the chromatogram developed with light petroleum containing successively smaller

proportions of benzene. This furnished a very satisfactory chromatogram showing a 70 cm. uniform zone of β -carotene with a small deep red upper carotenoid zone (> 2 mm.) and a small amount of ψ -carotene which was discarded. The main zone was eluted with a mixture of ether-benzene-ethanol (7:2:1) which commenced to deposit crystals of carotene almost immediately after it had percolated from the column. The percolate was kept overnight at 0° , the crystals filtered off and the residue obtained by evaporation of the filtrate crystallized from acetone-benzene. The whole of the carotene thus obtained (58 g.) was thrice recrystallized from acetone-benzene, a 1 in. Hirsch funnel being used to remove traces of alumina, filter paper threads, etc.

Finally, 28 g. of this carotene were dissolved in boiling thiophene-free benzene (290 ml.) under CO_2 and the solution was gradually diluted with boiling acetone (4.5 l., freshly distilled in CO_2). Crystallization was continued overnight in an atmosphere of CO_2 . The crystals were filtered, washed in turn with freshly distilled acetone, ethanol, and light petroleum, and dried in a vacuum desiccator over P_2O_5 .

This sample of β -carotene melted sharply at 183.5° (uncorr., open melting-point tube) and showed no optical activity in benzene.

The spectroscopic characteristics, determined on a Hilger-Nutting spectrophotometer with calibrated apparatus, were as shown in Table 1.

Table 1

Solvent	Wave-length of main band (m μ)	$E_{1\text{ cm.}}^{1\%}$
Cyclohexane	456	2480
Chloroform	465	2300
Hexane	453	2530
Benzene	465	2350

The solvents were purified as follows:

Cyclohexane. Commercial sample, treated with fuming sulphuric acid at 10° , washed until neutral, dried and distilled.

Chloroform. Commercial sample, washed with alkali, dried and redistilled shortly before use; stabilized with 0.5% ethanol.

Hexane. Commercial sample fractionated and collected over a range of 0.6° .

Benzene. Commercial thiophene-free sample fractionated and collected over a range of 0.2° .

In the cases of hexane and cyclohexane it was necessary to warm the solvent to effect complete solution of the carotene.

The absorption curve in cyclohexane was determined on a Hilger E498 spectrograph with Spekker photometer (Fig. 1).

The antimony trichloride reaction showed a maximum at $590\text{ m}\mu$ with an $E_{1\text{ cm.}}^{1\%}$ value of 460 (cf. Hunter & Scott, 1941).

A 2 g. sample of the β -carotene was further recrystallized four times from acetone-benzene and once from carbon disulphide-ethanol. This had m.p. 184° (initial bath temperature 180°). The spectroscopic values of this specimen are compared with those recorded in more recent literature as set out in Table 2.

Table 2

Solvent	Wave-length of main band (m μ)	$E_{1\text{ cm.}}^{1\%}$	Literature
Cyclohexane	456	2490	2400 (456 m μ)*
Chloroform	465	2370	2200 (463 m μ)† 2310 (463 m μ)‡
Hexane	453	2610	2580 (450 m μ)§ 2540 (450 m μ in petrol) 2500 (452 m μ in petrol)†
Benzene	465	2370	—

* Braude, Foot, Henry, Kon, Thompson & Mead (1941).

† Gillam (1935).

‡ Hunter & Scott (1941).

§ Zscheile, White, Beadle & Roach (1942).

|| Strain (1939).

Smakula (1934) recorded a value of 2760 at $450\text{ m}\mu$ in hexane but no details of the preparation or source of the specimen are given.

SUMMARY

1. The preparation of a relatively large sample of β -carotene of a high degree of purity is described.
2. The ultra-violet absorption spectrum of a fresh solution of this carotene in cyclohexane showed no inflexion at $330\text{--}340\text{ m}\mu$.
3. Further purification by five recrystallizations brought about little change in the spectroscopic values. The final figures were all appreciably higher than those recorded in the recent literature.

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