5. No phosphorylation of gluconate by kidney homogenate could be detected under conditions where glucose was readily phosphorylated.

I would like to thank Professor F. Dickens, F.R.S., for his constant interest and advice and Dr J. G. Bennette for providing the mouse sarcoma.

REFERENCES

- Albaum, H. G. & Umbreit, W. W. (1947). *J. biol. Chem.* **167**, 369.
- Andersson, B. (1934). *Hoppe-Seyl. Z.* **225**, 57.
- Brown, S. C. & Miller, W. W. (1947). *Rev. sci. Instrum.* **18**, 496.
- Calvin, M., Heidelberger, C., Reid, J. C., Tolbert, B. M. & Yankwich, P. E. (1949). *Isotopic Carbon*, p. 76. London: Chapman and Hall Ltd.
- Cohen, S. S. & McNair Scott, D. B. (1950). *Nature, Lond.*, **166**, 781.
- Colowick, S. P. & Kalckar, H. M. (1943). *J. biol. Chem.* **148**, 117.
- De Ley, J. & Cornut, S. (1951). *Nature, Lond.*, **168**, 515.
- Dickens, F. & Glock, G. E. (1951). *Biochem. J.* **50**, 81.
- Dische, Z. & Borenfreund, E. (1951). *J. biol. Chem.* **192**, 583.
- Entner, N. & Stanier, R. Y. (1951). *J. Bact.* **62**, 181.
- Euler, H. von, Adler, E., Schlenk, F. & Günther, G. (1935). *Hoppe-Seyl. Z.* **233**, 120.
- Fischer, O. L. H. & Taube, C. (1927). *Ber. dtsch. chem. Ges.* **60**, 1704.
- Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
- Hagedorn, H. C. & Jensen, B. N. (1923). *Biochem. Z.* **137**, 92.
- Harrison, D. C. (1931). *Biochem. J.* **25**, 1016.
- Hassid, W. Z., Doudoroff, M., Barker, H. A. & Dore, W. H. (1946). *J. Amer. chem. Soc.* **68**, 1465.
- Hillemann, H. (1938). *Ber. dtsch. chem. Ges.* **71**, 34.
- Horecker, B. L. & Kornberg, A. (1948). *J. biol. Chem.* **175**, 385.
- Hough, L., Jones, J. K. N. & Wadman, W. H. (1950). *J. chem. Soc.* p. 1702.
- Krebs, H. A. (1933). *Hoppe-Seyl. Z.* **217**, 191.
- Lanning, M. C. & Cohen, S. S. (1951). *J. biol. Chem.* **189**, 109.
- LePage, G. A. & Mueller, G. C. (1949). *J. biol. Chem.* **180**, 975.
- Lugg, J. W. H. & Overell, B. T. (1948). *Aust. J. sci. Res., Ser. A*, **1**, 98.
- Mejbaum, W. (1939). *Hoppe-Seyl. Z.* **258**, 117.
- Moore, S. & Link, K. P. (1940). *J. biol. Chem.* **133**, 293.
- Partridge, S. M. (1949). *Nature, Lond.*, **164**, 443.
- Slater, E. C. (1950). *Biochem. J.* **46**, 484.
- Stetten, M. R. & Stetten, De W. (1950). *J. biol. Chem.* **187**, 241.
- Stetten, M. R. & Topper, Y. J. (1953). *J. biol. Chem.* **203**, 653.
- Straub, F. B. (1940). *Biochem. J.* **34**, 483.
- Strecker, H. J. & Korkes, S. (1952). *J. biol. Chem.* **196**, 769.
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1949a). *Manometric Techniques*, p. 119. Minneapolis: Burgess Publishing Co.
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1949b). *Manometric Techniques*, p. 17. Minneapolis: Burgess Publishing Co.
- Van Slyke, D. D. & Folch, J. (1940). *J. biol. Chem.* **136**, 509.

The Carotenoids of the Petals of Two Species of *Tecoma*

By M. M. TAHA

Department of Biochemistry, Faculty of Medicine, Alexandria

(Received 29 April 1954)

Two species of the tropical *Tecoma* trees, belonging to the Bignoniaceae family, carry flowers of different colour. Those of *T. stans* are yellow, whilst those of *T. capensis* are deep red. Such variation in colour between two species of the same botanical family stimulated the study of the distribution of their carotenoid pigments, especially as no flowers of other members of the Bignoniaceae have been previously investigated, although Zechmeister & Sandoval (1945) demonstrated the presence of the colourless polyene phytofluene in species of *Bignonia*.

METHODS

Extraction of pigments. Freshly collected flowers (50 g.) were chopped with a sharp knife and heated with ethanolic KOH (300 ml., 10%, w/v) for 10 min. at 70° and then left overnight under N₂ at room temperature. The pigments were

extracted according to the method of Goodwin & Taha (1950) and dissolved in light petroleum (except where otherwise stated light petroleum b.p. 40–60° was used). Sometimes a few drops of ethanol were added before the light petroleum to facilitate the dissolution of xanthophylls. After standing overnight under N₂ in the refrigerator, the extract was filtered from a white residue, and examined as described below.

Fractionation of pigments. The pigments were separated into epiphasic and hypophasic fractions by partition between light petroleum and 90% (v/v) aqueous methanol, and each fraction was chromatographed separately. The monohydroxy carotenoids were assumed to be in the epiphasic fraction.

Chromatography. The pigments were separated on columns of either activated alumina (British Drug Houses Ltd.) or alumina deactivated by the method of Goodwin & Taha (1950). Alumina was also weakened by making a paste of it with ethanol and spreading out the paste in layers about an

inch thick in wide porcelain dishes. These were dried in the oven at 60°, with thorough mixing at intervals, until a faint odour of ethanol remained in the powder.

Identification of pigments. The criteria used for identifying the pigments were spectroscopic examination, mixed chromatography, relative adsorptive power and phase test.

Quantitative determination. Each pigment fraction was dissolved in a known volume of light petroleum, and the extinction of the solution was measured at its wavelength of maximal absorption. The amount of the pigment was then calculated by using its $E_{1\text{ cm}}^{1\%}$, λ_{max} , as reported by Goodwin (1952a). In the case of the pigment assumed to be rubixanthin-like, $E_{1\text{ cm}}^{1\%}$ for γ -carotene was applied, since the absorption spectra of both pigments are similar.

RESULTS

Tecoma stans

Epiphasic fraction. This showed on chromatography a single orange band, which after purification exhibited absorption maxima at 428, 452 and 480 m μ . in light petroleum, identical with those of β -carotene; this was proved to be β -carotene by the mixed chromatogram test.

The hypophasic fraction. A single strongly adsorbed broad yellow band was obtained from this fraction. It showed an absorption curve almost the same as that of zeaxanthin with λ_{max} 424, 447 and 473 m μ . A mixture of this pigment and an authentic preparation of zeaxanthin obtained from *Zea mays* could not be separated chromatographically.

The concentration of total carotenoids was 12.6 mg./100 g. fresh flowers, and of this 1.8 mg. were epiphasic and 10.8 mg. hypophasic. Zeaxanthin is thus the more abundant pigment present in *T. stans*.

Tecoma capensis

Epiphasic fraction. Using a column of active alumina, four coloured zones were separated; in addition to these, a small amount of the colourless polyene phytofluene was found in the colourless eluate running just before the least strongly adsorbed pigment. It was identified as phytofluene by its blue-green fluorescence in u.v. light and absorption maxima at 330, 348 and 368 m μ . in light petroleum. This colourless polyene was absent from *T. stans*.

Pigment 1. (The least strongly adsorbed band.) This pigment, which occurred in small amounts, showed maxima at 428, 452 and 480 m μ . in light petroleum, and was identified as β -carotene in the manner described for *T. stans*.

Pigment 2. This red pigment, found in greater amounts than β -carotene, was separated from pigment 3, with which it was closely associated on the column, by using a developer of light petroleum containing 5% (v/v) diethyl ether. After purification by repeated chromatography, it showed absorption maxima at 438, 464 and 494 m μ . in light

petroleum, identical with those of γ -carotene. This was not confirmed further, because the unique spectrum of γ -carotene together with its adsorptive power renders it unmistakable.

Pigment 3. This red pigment, which formed a larger band than that of γ -carotene, was identified as lycopene in three ways: (a) by the shape of its absorption curve in light petroleum with maxima at 447, 474 and 506 m μ ., which was identical with that of pure lycopene; (b) by its characteristic red colour on the column and its position above both γ -carotene and β -carotene; (c) by a mixed chromatogram with an authentic preparation of lycopene obtained from fresh tomatoes.

Pigment 4. (The most strongly adsorbed zone.) On partition between light petroleum and 95% (v/v) aqueous methanol, this deep orange pigment remained epiphasic, but with 97% (v/v) aqueous methanol it distributed itself between both layers, an indication that it possessed one OH group; its location on the chromatogram above lycopene supported this view. When, however, its absorption maxima (434, 462 and 490 m μ . in light petroleum b.p. 70–80°) were compared with those of the monohydroxy carotenoids, cryptoxanthin, lycopexanthin and rubixanthin, they corresponded most closely to those of rubixanthin. Its absorption spectrum (Fig. 1), which was unchanged by repeated chromatography of the pigment, was much less well defined than that of γ -carotene, from which that of rubixanthin is said to be indistinguishable (see Goodwin, 1952b). This rubixanthin-like pigment may not be the same as the original rubixanthin of Kuhn & Grundmann (1934). Goodwin (1954) has recently found a similar pigment in the petals of *Calendula officinalis*.

Fig. 1. The absorption spectra of the rubixanthin-like pigment, ---, and of γ -carotene, —, in light petroleum. The concentrations of the two pigments were adjusted so that E_{max} was the same for each solution.

Table 1. *The quantitative distribution of the carotenoids in the flowers of Tecoma capensis*

Pigment	Amount (mg./100 g. fresh wt.)
β -Carotene	2.2
γ -Carotene	4.3
Lycopene	5.9
Rubixanthin-like pigment	7.3
Total epiphasic pigments	19.7
Zeaxanthin	8.9
Total pigments	28.6

The hypophasic fraction. Zeaxanthin was the only pigment found.

Quantitative experiment. The amounts of the pigments in *T. capensis* are given in Table 1.

DISCUSSION

Goodwin (1950, 1952b) stated that flowers contain less carotenoids than the corresponding leaves, but that flower carotenoids are more specific to plant species than are leaf carotenoids. This appears to be true in the case of the petals of the two species of *Tecoma* investigated. Although the corresponding amounts of β -carotene and zeaxanthin in the two species do not differ appreciably, *T. capensis* petals contain much more of the red pigments lycopene and γ -carotene and the deep orange rubixanthin-like pigment. This not only results in the total amount of carotenoids in *T. capensis* being 2.3 times that of *T. stans*, but alters the ratio of epiphasic to hypophasic pigments from 1:6 in *T. stans* to 2.2:1 in *T. capensis*.

Epoxy-carotenoids are widely distributed in blossoms (Goodwin, 1952b), but in the case of the two species of *Tecoma* none of these epoxides was detected; zeaxanthin was, however, found in abundance. The difference in colour between the yellow flowers of *T. stans* and the red flowers of *T. capensis* is due to the presence of lycopene, γ -

carotene and the rubixanthin-like pigment in the latter. In *C. officinalis* the red varieties contain much more lycopene than the yellow varieties (Zechmeister & Cholnoky, 1932).

SUMMARY

1. The carotenoids of the flowers of two species of *Tecoma*, *T. stans* and *T. capensis*, have been investigated.

2. Both species contain β -carotene and zeaxanthin, whilst *T. capensis* contains in addition γ -carotene, lycopene, a rubixanthin-like pigment and a trace of the colourless polyene phytofluene.

3. The concentration of carotenoids in the red flowers of *T. capensis* and the yellow flowers of *T. stans* are 28.6 and 12.6 mg./100 g. fresh weight respectively. The corresponding ratios of epiphasic to hypophasic pigments in the two species are 2.2:1 and 1:6.

I wish to express my thanks to Dr T. W. Goodwin of the Biochemistry Department, Liverpool University, for his valuable advice, and to Professor A. Nasr and Dr Shokry I. Saad of the Botany Department, Faculty of Science, Alexandria, for their help in the identification of the *Tecoma* species and for the supply of the flowers of *T. capensis*. I am also indebted to Professor B. Flaschenträger for his encouragement and supervision.

REFERENCES

- Goodwin, T. W. (1950). *Biol. Rev.* **25**, 391.
- Goodwin, T. W. (1952a). *Biochem. J.* **51**, 458.
- Goodwin, T. W. (1952b). *The Comparative Biochemistry of the Carotenoids*. London: Chapman and Hall.
- Goodwin, T. W. (1954). *Biochem. J.* **58**, 90.
- Goodwin, T. W. & Taha, M. M. (1950). *Biochem. J.* **47**, 244.
- Kuhn, R. & Grundmann, C. (1934). *Ber. dtsch. chem. Ges.* **67**, 339, 432, 1133.
- Zechmeister, L. & Cholnoky, L. (1932). *Hoppe-Seyl. Z.* **208**, 27.
- Zechmeister, L. & Sandoval, A. (1945). *Arch. Biochem.* **8**, 425.

The Purification and Properties of Horse Liver Esterase

By JANE BURCH

*Department of Biochemistry, University of Cambridge, and Department of Biochemistry, University of Leeds**

(Received 7 December 1953)

When this work was begun in 1948 an extensive literature on the liver esterases existed, but very little purification had been carried out. It was known that esterase activity was not readily extracted from fresh liver, and prolonged autolysis (Bamann, Mukherjee & Vogel, 1934) or drying with

acetone followed by extraction of the powder with dilute alkali (Willstätter & Memmen, 1924) had been used to prepare active solutions. Many recorded observations on the esterases (reviewed by Ammon & Jaarma, 1950) seemed to be most readily explained by the presence of two or more enzymes in the preparations; and more recent work has shown repeatedly that more than one enzyme hydrolysing

* Present address.