Phytochemistry, 1977, Vol 16, pp. 612–613. Pergamon Press Printed in England

RHODOXANTHIN, THE RED PIGMENT OF *EQUISETUM ARVENSE* SPOROPHYTES

MATTHEW J. CYRONAK*, GEORGE BRITTON† and KENNETH L. SIMPSON*

*Department of Food Science and Technology, University of Rhode Island, Kingston, Rhode Island 02881;
†Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, England.

(*Received* 12 *November* 1976)

Key Word Index—*Equisetum arvense*; Pteridophyta; horsetail, carotenoid; rhodoxanthin.

It has long been known [1] that the gametophytes of *Equisetum* spp. (horsetail) are characterized by a yellow or pinkish coloration due to the presence of a red pigment, apparently in lipoidal droplets in the plastids [2]. A similar red pigment has also been detected in vegetative stems of several *Equisetum* spp. [3–5]. Early reports suggested that the red pigment was rhodoxanthin 4′,5′-didehydro-4,5′-*retro*-β,β-carotene-3,3′-dione) [3–6]. However, in more recent work [2], the red pigment from gametophytes of *E. hyemale* and *E. arvense*, from overwintering stems of *E. hyemale* and from young vegetative stems of *E. arvense* was identified as being similar to, but not identical with, rhodoxanthin. The identity of the red pigment from *E. arvense* sporphytes has therefore now been reinvestigated.

RESULTS AND DISCUSSION

The red pigment (1.82 μg/g dry wt) isolated from the sporophytes of *E. arvense* constitutes approximately 0.57% of the total carotenoids (319.2 μg/g dry wt) of the plant. The red pigment exhibited TLC and visible light absorption properties identical to those of authentic rhodoxanthin from fruits of *Taxus baccata*. $NaBH_4$ reduction of the *E. arvense* pigment (in EtOH) confirmed the presence of two carbonyl groups, and gave a main product identical (visible light spectrum, co-chromatography) to eschscholtzxanthin, (4′,5′-didehydro-4,5′-*retro*-β,β-carotene-3,3′-diol) the product of $NaBH_4$ reduction of the rhodoxanthin. This identification was confirmed by the MS of the *E. arvense* pigment and its $NaBH_4$ reduction product being identical to those of rhodoxanthin and eschscholtzxanthin respectively.

Contrary to a previous report, therefore, the red pigment isolated from *E. arvense* is identified, unambiguously, as rhodoxanthin. The specificity of the occurrence of rhodoxanthin in *Equisetum* gametophytes in association with developing antheridia [2] is striking. This apparent relationship of the carotenoid with male sex development is worthy of further investigation.

EXPERIMENTAL

Plant material. Samples of *E. arvense* were collected locally at the University of Rhode Island during the months of May and June. The mature vegetative stems of the sporophyte were picked prior to each analysis and the nodes and rhizomes, which exhibited a dark purplish coloration, were excised and used in the extraction.

Pigment extraction and purification. The freshly excised nodes and rhizomes were macerated with Me_2CO–petrol (bp 30–50°) and the Me_2CO was quickly washed out of the extract and the petrol removed under vacuum. The wet residue was saponified overnight (10% KOH in EtOH) in the dark under N_2 at room temp. The saponified extract was transferred to petrol, washed with H_2O, dried (Na_2SO_4), concentrated under vacuum and chromatographed on a column of MgO: HyfloSuper Cel (1:2, w/w) with 8% Me_2CO in petrol as the developing solvent. The strongly adsorbed cherry-red band was eluted from the extruded adsorbent with Me_2CO, and rechromatographed by preparative TLC on Si gel G (5% MeOH in C_6H_6). The cherry-red band (R_f 0.8) was rechromatographed on MgO–Kieselguhr G (1:1, w/w) [7] with Me_2CO–C_6H_6 (2:3) as the developing solvent. The red pigment had R_f 0.56, λ_{max} in petrol at (460), 480, (506) nm, in C_6H_6 at (475), 497, (526) nm and in 95% EtOH at 495 nm; MS-M^+ 562 (100%, $C_{40}H_{50}O_2$), fragment ions at *m/e* 470 (15% M-92), 456 (26%, M-106), 425 (5%, M-137), 399 (6%, M-163) 359 (7%, M-203). The MS is identical to that of authentic rhodoxanthin.

$NaBH_4$ reduction. The isolated red pigment was reduced with $NaBH_4$ in EtOH [7] and the products chromatographed on Si gel G (5% MeOH in C_6H_6). The isolated orange pigment had R_f 0.23, λ_{max} in petrol at (442), 462, 492 nm, in C_6H_6 at (455), 477, 508 nm and in 95% EtOH at (477), 467, 496 nm; MS—M^+ 566 (14%, $C_{40}H_{54}O_2$) fragment ions at *m/e* 548 (26%, M-H_2O), 515 (24%, 530-CH_3), 474 (6%, M-92), 460 (6.5%, M-106), 456 (5%, 548-92), 442 (5%, 148-106), 438 (18%, 530-92), 424 (19%, 530-106). The MS was identical to that of eschscholtzxanthin produced by $NaBH_4$ reduction of authentic rhodoxanthin.

Co-chromatography. The isolated rhodoxanthin and its reduction product were co-chromatographed with authentic rhodoxanthin and eschscholtzxanthin on Si gel G and MgO–Kieselguhr G (1:1, 2/2) in the solvent systems described above.

Absorption spectra. Quantitative estimation of total carotenoids and of rhodoxanthin were made using $E^{1\%}_{1cm}$ value of 2500 (450 nm) and 2500 (479 nm) respectively [8].

Mass spectra. MS were determined by Mr. G. Harriman on an MS12 instrument. The direct insertion probe was used, and an ion source temperature 185° and ionizing voltage 70 eV.

Acknowledgements—Thanks are given to Dr. Richard W. Glass and Karen Bonatti for their assistance and to Hoffman-LaRoche for their gift of authentic rhodoxanthin. This work was supported by research grant OIP74-01414-A02 from the National Science Foundation. Rhode Island Agricultural Experiment Station Contribution No. 1717, University of Rhode Island, Kingston, R.I. 02881.

REFERENCES

1. Hofmeister, W. (1852) *Flora* **35**, 1.
2. Hauke, R. L. and Thompson, J. J. (1974) *Tiawania* **18**, 176.

3. Lippmaa, T. (1926) *Ber. Deuts. Bot. Ges.* **44**, 643.
4. Lippmaa, T. (1926) *Allgemeiner Teil. Acta Inst. et Horti. Bot. Univ. Tarteunsis* **1**, 1.
5. Lippmaa, T. (1926) *Spezialler Teil. Acta Inst. et Horti. Bot. Univ. Tartsunsis* **1**, 1.
6. Prat, S. (1924) *Biochem. Z.* **152**, 495.
7. Britton, G. and Goodwin, T. W. (1971) *Methods Enzymol.* **18C**, 654.
8. Davies, B. H. (1976) in *Chemistry and Biochemistry of Plant Pigments*, 2nd edition, Vol. 2 (Goodwin, T. W., ed.) p. 38. Academic Press, New York.

Phytochemistry, 1977, Vol 16, pp 613–614 Pergamon Press Printed in England

NATURAL β-APO-4′-CAROTENOIC ACID METHYL ESTER IN THE FUNGUS *VERTICILLIUM AGARICINUM*

L. R. G. VALADON and ROSEMARY S. MUMMERY
Department of Botany, Royal Holloway College, Egham Hill, Egham, Surrey, TW20 0EX, U.K.

(*Received* 30 *September* 1976)

Key Word Index—*Verticillium agaricinum*; fungi; neurosporaxanthin methyl ester; β-apo-4′-cartoenoic acid methyl ester.

Valadon and Mummery [1] isolated an unknown red band from the fungus *Verticillium agaricinum* which they called Red Band I. This carotenoid has been investigated further and on the following evidence was shown to be β-apo-4′-carotenoic acid methyl ester (**1**). It had the same absorption spectra in visible and infrared light and the same partition ratio as β-apo-4′-carotenoic acid methyl ester [2]. The hydride-reduced product of **1** and of synthetic β-apo-4′-carotenal (**2**) i.e. compound **3**, had the same visible and IR properties and could not be separated chromatographically. Further, when **1** was saponified it yielded β-apo-4′-carotenoic acid (**4**) identical in all respects to neurosporaxanthin isolated from the same fungus [3].

This is therefore the first demonstration of the methyl ester of neurosporaxanthin in the Fungi Imperfecti [4].

EXPERIMENTAL

V. agaricinum. Cultured as already described [3] and the carotenoids extracted by conventional methods and separated on an MgO–Celite (1:1) column. Red Band I was found below torulene on the column and purified further on TLC and on paper impregnated with Si gel [5].

Red Band I (**1**). Very similar absorption spectra in visible (~445, 473, 505 nm in *n*-hexane; 472, ~500 nm in MeOH) and IR light as β-apo-4′-carotenoic acid methyl ester [2]. When **1** was saponified using 10% KOH overnight, β-apo-4′-carotenoic acid (**4**) was obtained which could not be separated from neurosporaxanthin obtained from the same fungus [3]. Partition ratio of **1** in hexane–MeOH (5:95) was 93.5/6.5 whereas when it was converted to **4** the partition ratio changed to 1/9. R_f value of **1** on Si gel paper (SG 81) developed with Me_2CO–*n*-hexane (2:98) was 0.30. $LiAlH_4$-reduction of **1** in dry Et_2O resulted in the formation of β-apo-4′-carotenol (**3**) (~434, 459.5, 488 nm in *n*-hexane; ~431, 458, 487 nm in MeOH) whose partition ratio was 62/38. R_f value of **3** on Si gel paper (Me_2CO–*n*-hexane (2:98)) was 0.11.

Synthetic β-apo-4′-carotenal (**2**). When **2** (~450, 483, ~516 nm in *n*-hexane; 482, ~505 in MeOH), which had a partition ratio of 88/12, was subjected to $LiAlH_4$-reduction, the compound formed was β-apo-4′-carotenol. Further, when the hydride-reduced product of Red Band I (**3**) was oxidised with *p*-chloranil and I_2 in the presence of strong artificial light, the main product