Phytochemistry, 1971, Vol. 10, pp. 625 to 629. Pergamon Press. Printed in England.

THE OCCURRENCE OF PLECTANIAXANTHIN IN *CRYPTOCOCCUS LAURENTII*

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(*Received* 8 *April* 1970, *in revised form* 23 *May* 1970)

Abstract—Plectaniaxanthin, recently isolated from the mushroom *Plectania coccinea* (Scop. ex Fr.) Fuck., has now been isolated from the asporogenous yeast *Cryptococcus laurentii* (Kuff.) Skinner var. *flavescens* (Saito) Lodder et Kreger-van Rij. The identity of the compound was established by visible, NMR and high resolution mass spectra and by chemical reactions. Two further pigments have been isolated and preliminary data indicates that they have the same monocyclic dodecaene chromophore as plectaniaxanthin.

INTRODUCTION

The carotenoid pigments of the asporogenous yeasts, in particular the genera *Rhodotorula* and *Cryptococcus*, have been the subject of many reports.[1-6] These yeasts were found to contain only the hydrocarbon carotenoids and the carotenoid acid torularhodin. However, in the course of recent studies on the biosynthesis of acyclic carotenoids in *Rhodotorula mucilaginosa* and *R. aurantiaca*,[7-9] 3′,4′-dehydro-17′-hydroxy-γ-carotene and 3′,4′-dehydro-17′-oxo-γ-carotene (incorrectly labeled 18′) have been isolated and identified. The latter pigment had been previously found in an unidentified microorganism.[10] Accordingly it was proposed that these two compounds were intermediates during the transformation of torulene to torularhodin in these yeasts.[8]

This paper reports the isolation of the recently described xanthophyll plectaniaxanthin[11] (I) from *Cryptococcus laurentii* and its identification with the aid of an high resolution mass

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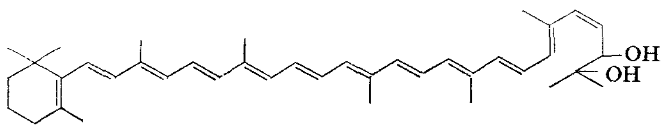
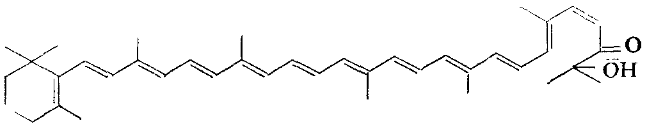
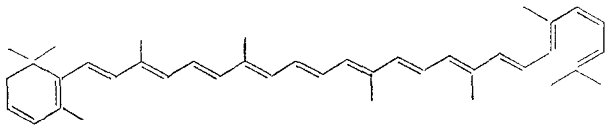
spectrum, an NMR spectrum and chemical reactions. In addition to plectaniaxanthin, preliminary data is presented for two further xanthophylls isolated from the yeast.



Plectaniaxanthin (I)



2′-Dehydroplectaniaxanthin (II)



3,4-Dehydrotorulene (III)

RESULTS AND DISCUSSION

Plectaniaxanthin crystallized from petrol and melted at 172–173° (uncorrected). The visible spectra of plectaniaxanthin in petrol (λ_{max} 445 471 502) and in acetone (λ_{max} 450 474 507) and the partition coefficient of 87:13 in petrol: 85% methanol closely resemble published values for plectaniaxanthin isolated from the mushroom *Plectania coccinea*.[11] The NMR spectrum confirmed the monocyclic nature of the compound. Signals at τ8 98 (C-1 geminal methyls, 6H) and 8·27 (C-5 methyl, 3H) established the β-ring endgroup; signals at τ8 82 and 8·75 (C-1′ geminal methyls, 6H) and 8·07 (C-5′ methyl, 3H) the acylic end of the compound. The NMR spectrum also showed a signal at τ8·02 (C-9, C-13, C-9′, C-13′ in-chain olefinic methyls, 12H). The signals were of the correct relative intensities.

The high resolution mass spectra of the main fraction of the sample (150–260°) are in good agreement with the structure of plectaniaxanthin (I) and its low resolution spectrum reported by Enzell *et al.*[12] The calculated molecular ion mass from four runs ranged from 568 4280 to 568·4289 corresponding to an elemental composition of $C_{40}H_{56}O_2$, which has a mass of 568 4266 (Table 1). This measurement is supported by C^{13} isotope peak of proper intensity at *m/e* 569·4332. The proposed structure is supported further by peaks corresponding to the loss of C_7H_8 and C_8H_{10}. The presence of one or more alcohol groups is indicated by the loss of water and the loss of an isopropanol fragment. The assignment of the OH groups to adjacent carbon atoms is supported by the peak at 534·4214 which has the composition of $C_{40}H_{54}$ and results from the loss of H_2O_2. Further support to the contention of adjacent OH groups is given by the presence of a series of low molecular weight fragments with two oxygen atoms.

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TABLE 1. CHARACTERISTIC FRAGMENTS FROM HIGH RESOLUTION MASS SPECTRUM OF PLECTANIAXANTHIN

Intensity	*m/e* Measured	*m/e* Calculated	Formula	Remarks
0·56	568·4289	568·4266	$C_{40}.H_{56}.O_2$	Mol-Ion
0·12	550·4217	550·4161	$C_{40}.H_{54}.O$	Mol-Ion − H_2O
0·10	534·4214	534·4212	$C_{40}.H_{54}$	Mol-Ion − H_2O_2 (or Torulene Imp.)
0·13	476·3662	476·3642	$C_{33}.H_{48}.O_2$	Mol-Ion − C_7H_8 (Toluene)
2·42	462·3453	462·3486	$C_{32}.H_{46}.O_2$	Mol-Ion − C_8H_{10} (Xylene)
0·26	444·3416	444·3381	$C_{32}.H_{44}.O$	Mol-Ion − $C_8H_{12}O$ (Xylene + water)
0·28	403·3052	403·2991	$C_{29}.H_{39}.O$	Mol-Ion − $C_{11}H_{17}O$ (Xylene + $(CH_3)_2COH$)
0·14	402·2963	402·2913	$C_{29}.H_{38}.O$	Mol-Ion − $C_{11}H_{18}O$ (Xylene + $(CH_3)_2CHOH$)
0·32	370·2908	370·2862	$C_{25}.H_{38}.O_2$	Mol-Ion − $C_{15}H_{18}$ (Xylene + Toluene)
0·11	352·2802	352·2757	$C_{25}.H_{36}.O$	Mol-Ion − $C_{15}H_{20}O$ (Xylene + Toluene + water)
0·22	73·0297	73·0288	$C_3.H_5.O_2$	
0·26	86·0369	86·0366	$C_4.H_6.O_2$	

p-Chloranil oxidation of plectaniaxanthin gave 2′-dehydroplectaniaxanthin (II) which cochromatographed with the synthetic compound on Chromagram silica gel sheets. Conversely authentic 2′-dehydroplectaniaxanthin was reduced with $LiAlH_4$ to form plectaniaxanthin[13] which cochromatographed with the isolated compound. The R_f for 2′-dehydroplectaniaxanthin was 0·90 compared to an R_f of 0·36 for plectaniaxanthin in the same system. Furthermore, allylic dehydration of plectaniaxanthin produced 3,4-dehydrotorulene (III), a non-polar compound with an elongated chromophore.[14]

During the isolation of plectaniaxanthin two additional pigments were separated having similar visible spectra to plectaniaxanthin indicating the monocyclic dodecaene chromophore contained by β-apo-2′-carotenol (C_{37}).[15] The low resolution mass spectrum of the least polar of the two compounds gave a molecular ion at *m/e* 552 which suggests a monol. Evidence for the allylic nature of the hydroxyl group and its probable position was derived from the facile dehydration of the compound with acid chloroform. The dehydration product was identical to torulene. This suggested that the hydroxyl group is located at the 2′ position. Further tentative support for the position of the OH group was evident in the NMR spectrum, although the sample analyzed contained some impurities and the amount isolated precluded further purification by recrystallization. The signals due to the C-1′ geminal methyls were non-equivalent and were split into doublets centered at τ8·77 ($J = 5$Hz) and 8·73 ($J = 5$Hz). This suggests a structure of 2′-hydroxy-1′,2′-dihydrotorulene. It is interesting to note that Bonaly[9] described a similar compound isolated from a UV mutant of *Rhodotorula mucilaginosa* and suggested that the hydroxyl group was located in the 1′ position. Further work is continuing on the full identification of the two pigments.

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From our results it does appear that in the yeasts of the genera *Cryptococcus* and *Rhodotorula* torulene may be also transformed to several interesting hydroxydihydrotorulenes. These pigments comprise over half the total pigment content of the yeast after 7 days of shaking culture. They have also been isolated from *Rhodotorula aurantiaca* (Saito) Lodder C.B.S. 317* and from *Cryptococcus hungaricus* (Zsolt) Phaff et Fell syn. (*Dioszegia hungarica* Zsolt) C.B.S. 4214.*

EXPERIMENTAL

Materials and Methods

The reagents and solvents were of analytical grade. Column chromatography was carried out on Whatman CF 11 fibrous cellulose powder and Microcel-C. Chromagram sheets (No. 6060) composed of 100 μ layers of silica gel were used for TLC and were developed with EtOAc–CH_2Cl_2 (1:4).

NMR spectra were measured at 60 MHz and were determined in $CDCl_3$ relative to the internal standard tetramethylsilane. High resolution mass spectra were recorded on an AEI type MS 902 mass spectrometer with a direct inlet system. The spectra were obtained at a source pressure of 2×10^{-5} torr and 500 μ-amps anode current at an accelerating voltage of 8 kV. The temperature of the sample was raised from 35° to 500° over a period of 45 min in an approximately linear fashion. Spectra were recorded at various temperatures as shown in Table 2. Each spectrum was recorded from $m/e = 920$ to $m/e = 52$ at a resolution of $M/\Delta M - 10{,}000$ at a rate of 40 sec/decade. Perfluorokerosine was used as a mass marker and mass measurement of all peaks was carried out by means of our computerized data analysis system.[16] The mass spectra which were taken at temperatures of 400° and 500° showed the presence of at least three impurities of high molecular weight (Table 2).

Further evidence for structural elucidation was derived from several diagnostic chemical reactions. They were partition tests,[17] reduction with $LiAlH_4$ in dry ether,[18] allylic oxidation with *p*-chloranil[19] and allylic dehydration with acid chloroform.[20]

TABLE 2. COMPOUND DISTRIBUTION AGAINST PROBE TEMPERATURE

Temperature	Total ion current	Compound
35°	0	*PFK*
60°	100	Trace of carotenoid
110°	400	
150°	1200	*Plectaniaxanthin*
190°	3000	
225°	1500	
260°	1400	
400°	2000	A carotenoid compound of mol. wt. 612·3381 and composition $C_6H_{44}O$
500°	3500	Tentatively identified were 3 compounds and/or fragments thereof: $C_{46}H_{44}O$, $C_{45}H_{50}O_4$ (654·3696), $C_{55}H_{56}O$ (732·4312)

Organism

Cryptococcus laurentii (Kuff.) Skinner var. *flavescens* (Saito) Lodder et Kreger-van Rij (C.B.S. 4256) was kindly provided by Dr. H. J. Phaff, Department of Food Science and Technology, University of California, Davis, U.S.A.

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Cultural Conditions

The yeast was maintained on malt agar slopes (5% malt extract) at room temp. (20–23°). The yeast was grown in shaking culture at room temp. in a medium containing 5% glucose and 0·5% yeast autolysate. Due to the slow growth of the yeast an innoculum was prepared as follows. The yeast was grown for several days on a fresh malt agar slope and then transferred to a 125 ml flask containing 50 ml of medium. After 4 days of growth the culture was transferred to a 1 l. flask containing 300 ml of medium and grown for a further 2 days. At this point the innoculum was split between 4 × 1 l. flasks containing 250 ml of medium and grown for 7 days.

Extraction and Purification of Pigments

The yeast cells were harvested by centrifugation, washed twice with distilled water and extracted immediately or stored at −20° until required. The cells were broken in acetone under nitrogen with a Braun MSK cell disintegrator at 4000 rev/min for 2 min cooled by liquid CO_2. The cell debris was centrifuged off and extracted with acetone until colorless. The pigments were transferred to petroleum ether dried and saponified by the usual method.[21] *Pl. coccinea* xanthophylls plectaniaxanthin and 2′-dehydroplectaniaxanthin are esterified,[11] the latter with linoleic acid.[22] The yeast xanthophylls are also esterified but the ester has not yet been fully characterized. Torularhodin was removed at this step and the unsaponifiable material was chromatographed on a column of Whatman CF 11 cellulose powder. Hydrocarbons, monohydroxy xanthophylls and sterols were eluted with 1% acetone in petroleum ether. Plectaniaxanthin was eluted with 10% acetone in petroleum ether and further purified on a Microcel-C column developed with 15% acetone in petroleum ether. The purity of the compound was confirmed on Chromagram silica gel sheets.

Acknowledgements—The authors would like to thank Hoffman-La Roche & Co. Ltd., Basle, Switzerland, for the generous gift of synthetic 2′-dehydroplectaniaxanthin. The work was supported by grant AM 11665 from U.S. Public Health Service to C.O.C. This paper is contribution No. 1367 of the University of Rhode Island Agricultural Experiment Station.

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