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cis- AND *trans*-PHYTOENE IN *VERTICILLIUM AGARICINUM*

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Abstract—*cis*-Phytoene and *trans*-phytofluene were identified in illuminated cultures of *Verticillium agaricinum* in addition to the other carotenoids already found to be present, namely: β-, β-zea-, ζ-, γ-carotene, torulene and neurosporaxanthin. *trans*-Phytoene was only identified in 9-fluorenone inhibited cultures in the light, even though the following inhibitors were used: 4-acetyldiphenyl, diphenylamine and benzophenone. Results obtained are not in disagreement with the suggestion that trans-phytoene may be formed first and not that the C_{40} compound arises by stereomutation.

INTRODUCTION

A POSSIBLE mechanism for *cis*- and *trans*-phytoene biosynthesis has been proposed by Goodwin.[1] *cis*-Phytoene has been isolated in a number of higher plants and micro-organisms[2] and would seem to be the natural compound. The *cis*-bond in phytoene may be preserved at least to the ζ-carotene stage,[2] after which presumably the all-*trans* forms appear. Recently, however, a number of micro-organisms[3,4] have been shown to contain *trans*-phytoene and *trans*-phytofluene which may suggest that the two pathways are going on side by side. *Mucor hiemalis* is the only fungus so far in which *trans*-phytoene and *trans*-phytofluene have been positively identified in inhibited cultures.[4] The fungus produces fairly large amounts of carotenoids (mostly phytoene and β-carotene) in the dark which are increased by about three times in the light. *Verticillium agaricinum*, on the other hand, is much more light sensitive and produces large amounts of carotenoids in the light but only traces in the dark. Different photoregulation mechanisms are presumably involved in the two fungi and an investigation of phytoene biosynthesis in *V. agaricinum* might reveal differences between the two organisms and perhaps help one to have a better insight into photoregulation in fungi.

RESULTS

Valadon and Mummery[5] have shown that *V. agaricinum* contained the following carotenoids: β-, β-zea, γ- and ζ-carotene, torulene and neurosporaxanthin, while the C_{40} colourless polyenes were not investigated due to insufficient material. In the present investigation the fungus was grown in light and in dark in liquid culture and it was possible to identify in the light grown cultures, *cis*-phytoene, *trans*-phytofluene as well as the other

[1] T. W. GOODWIN, *Carotenoids*, 588, Birkhäuser, Basel (1971).
[2] B. C. L. WEEDON, *Carotenoids*, 268, Birkhäuser, Basel (1971).
[3] O. B. WEEKS, A. G. ANDREWES, R. O. BROWN and B. C. L. WEEDON, *Nature, Lond.* **224**, 879 (1969).
[4] R. HERBER, B. MAUDINAS and J. VILLOUTREIX, *Compt. Rend.* **274**, 327 (1972).
[5] L. R. G. VALADON and R. S. MUMMERY, *Microbios.* **4**, 227 (1971).

pigments already observed. Two other bands *X* and *Y* were also obtained which had an absorption spectrum fairly similar to that of torulene and which are under further investigation. *cis*-Phytoene (47·1 μg/g dry wt) is the most abundant C_{40} polyene after 4 days growth, followed closely by γ-carotene (31·9 μg/g), neurosporoxanthin (30μg/g) and torulene (23·9 μg/g). In the dark, the fungus synthesizes mainly *cis*-phytoene (35·7 μg/g dry wt after 5 days) with traces of other pigments (6·7 μg/g). *cis*-Phytoene is likely to be the natural compound here as no *trans*-phytoene was observed at all. In *Mucor hiemalis*, *trans*-phytoene was found as a small constituent (*2%*)[6] of total phytoene but this increased considerably in the presence of certain inhibitors.

Verticillium agaricinum was grown in media containing the following inhibitors at a concentration of 30 μg/ml:4-acetyldiphenyl, diphenylamine (DPA), benzophenone and 9-fluorenone. In a preliminary experiment, 4-acetyldiphenyl was shown to be very inhibitory and no growth of the fungus resulted. The results with the other three inhibitors showed that there was a small amount of *cis*-phytoene with DPA, approximately half that in the normal cultures and no coloured carotenoids at all. With benzophenone, there was an increase in *cis*-phytoene (to 100 μg/g), approximately double that of the normal cultures, some *trans*-phytofluene and a trace of other carotenoids. With 9-fluorenone, however, it was possible to show the presence of both *cis*- and *trans*-phytoene (226 μg/g and 62 μg/g dry wt respectively), with approximately six times the total phytoene of normal cultures. So, *trans*-phytoene was observed for the first time in cultures of *V. agaricinum*. No other C_{40} polyenes were observed. As both the two isomers of phytoene were found in the 9-fluorenone inhibited cultures, it was decided to study their relative amounts over a period of 3–5 days. The results show that total phytoene increases from 195·8 μg/g after 3 days to 419·2 μg/g dry wt after 5 days. The relative amounts of *trans*-phytoene increase from 20·9 to 41·8% after 5 days and this latter value is roughly intermediate between that of *Rhodopseudomonas spheroides* (20%) and *M. hiemalis* (70%).[4] Further, in 5-day-old cultures one also obtains other isomers of phytoene. These isomers termed 'intermediate fraction' by Granger *et al.*[7] may be a mixture of *cis*- and *trans*-isomers.

DISCUSSION

All *trans*-phytoene has been identified in *Flavobacterium dehydrogenans*,[3] yet Weedon[2] stated that the possibility cannot be excluded that stereomutation has occurred during isolation. Herber *et al.*[6] working under conditions where stereomutation was unlikely to occur identified *trans*-phytoene in their dark grown cultures. In the present investigation the same conditions were adhered to and a number of differences can be observed between the two fungi.

All *trans*-phytoene is found only in 9-fluorenone inhibited cultures of *V. agaricinum* and does not seem to be the natural compound here unless present either in too small amounts to be detected or in a bound form. On the other hand, *trans*-phytofluene is identified in the normal and in benzophenone inhibited cultures. This is in agreement with the suggestion that *trans*-phytoene is probably formed first by an enzymic reaction, then is found in a bound form which thus becomes desaturated to phytofluene.[6] So, it can be seen that even though differences exist between the two fungi the results obtained may be made to fit in a fairly broad hypothesis. It is probably also that some *cis*-phytoene may be converted to *trans*-phytoene by a photochemical isomerisation as was shown by Maudinas *et al.*[8]

[6] R. HERBER, B. MAUDINAS, J. VILLOUTREIX and P. GRANGER, *Biochim. Biophys. Acta* in press.
[7] P. GRANGER, R. HERBER, B. MAUDINAS and J. VILLOUTREIX, *J. Magn. Reson.* in press.
[8] B. MAUDINAS, R. HERBER and J. VILLOUTREIX, *Phytochem. Photobiol.* in press.

EXPERIMENTAL

The fungus *V. agaricinum* (Link) Corda[5] was cultured on the same medium and under the same conditions as those used by Herber *et al.*[6] Extraction and identification of carotenoids were carried out as already described.[5,6]

Very small amounts of phytofluene were obtained. The phytofluene was identified as *trans*-phytofluene by its absorption spectra 331, 348 and 368 nm in petrol. and by the ratio of its absorption at 348 to that at 368 which is reported to be 1·04 for all *trans*-phytofluene.[9]

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[9] B. K. Koe and L. Zechmeister, *Arch. Biochem. Biophys.* **41**, 236 (1952).