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SHORT COMMUNICATION

THE OCCURRENCE OF PHYTOENE 1,2-OXIDE AND RELATED CAROTENOIDS IN TOMATOES

G. BRITTON and T. W. GOODWIN

Department of Biochemistry, University of Liverpool, Liverpool L69 3BX

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Abstract—A series of carotenoids has been isolated from ripe tomato fruit. One of these compounds has been fully identified (reactions, u.v. spectrum, mass spectrum) as phytoene 1,2-oxide; the others may be similar epoxy derivatives of phytofluene, ζ-carotene and lycopene.

INTRODUCTION

THE CAROTENE hydrocarbons from a number of strains of tomatoes have been well characterized,[1] but the oxygenated derivatives have been neglected. By the use of counter-current distribution techniques, Curl[2] was able to demonstrate the presence of a large number of xanthophylls. Zechmeister and Pinckard[3] isolated an interesting compound, phytofluenol, which appeared to be a polar derivative of phytofluene. No systematic analysis of tomato xanthophylls has, however, been reported.

The availability of the unsaponifiable lipid material from 11 kg of ripe tomatoes prompted an investigation into the nature of some of the oxygenated carotenoids present in tomatoes.

RESULTS AND DISCUSSION

A chromatographic fraction of the unsaponifiable lipid material from tomatoes, eluted from a column of alumina with 10% diethyl ether in petroleum ether, contained a series of carotenoids with u.v. and visible absorption spectra similar to those of the hydrocarbons phytoene, phytofluene, ζ-carotene and lycopene. From this series, one compound (*A*) was purified by TLC, and had u.v. spectrum identical to that of phytoene (λ_{max} (light petroleum) at 275, 285, 296 nm), but was more polar. *A* did not form an acetate (therefore was not a primary or secondary alcohol), could not be saponified (therefore was not an ester), and was not reduced by $NaBH_4$ (therefore was not an aldehyde or ketone). The TLC behaviour of *A* was similar to that of mutatochrome (β-carotene 5,8-oxide, also present in the fraction) and of squalene 2,3-oxide. *A* was more polar than lycopene and less polar than β-cryptoxanthin. This chromatographic behaviour thus suggested that *A* might be an ether or epoxide.

The mass spectrum of *A* showed a parent ion, M^+ at *m/e* 560·4979 ($=C_{40}H_{64}O$), and fragment ions at *m/e* 491 (M-69), 475 (M-85), 423 (M-137), 407 (M-153), 355 (strong, M-205)

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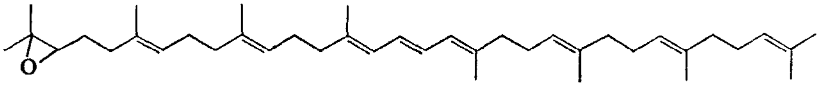
[1] R. J. H. WILLIAMS, G. BRITTON, J. M. CHARLTON and T. W. GOODWIN, *Biochem. J.* **104**, 767 (1967).
[2] A. L. CURL, *J. Food Sci.* **26**, 106 (1961).
[3] L. ZECHMEISTER and L. PINCKARD, *Experientia* **4**, 471 (1948).

and 339 (strong, M-221). Metastable ions were observed at *m/e* 225 (560 → 355), and at *m/e* 205·5 (560 → 339). No peak was observed at *m/e* 542 (M-H_2O).

The mass spectral data, and other properties of compound *A* are entirely consistent with structure I, i.e. phytoene 1,2-oxide (carotene numbering) analogous to the sterol precursor squalene 2,3-oxide. It is thought that the other members of the series may be similar epoxy derivatives of phytofluene, ζ-carotene and lycopene. Although 5,6-epoxy derivatives of cyclic carotenoids are well known (e.g. β-carotene epoxides, violaxanthin), this is the first example of naturally occurring epoxides of acyclic carotenoids.



(I)

No quantitative work has yet been carried out, but it appears that these epoxy derivatives are present in largest quantities in ripe fruits. They may represent the first stage in the oxidative breakdown of carotenoids in tomatoes.

EXPERIMENTAL

Materials

Normal red tomatoes (11 kg) were bought from a local shop.

Extraction and Purification Methods

Lipid material was extracted with acetone and saponified by standard procedures.[4] The unsaponifiable material was chromatographed on a column of neutral alumina (200 g, Brockmann Grade III). Hydrocarbons were eluted with 20% (v/v) benzene in petroleum ether (*B/P*), and polar materials then removed with 5% (v/v) ethanol in diethyl ether. This polar fraction was rechromatographed on a column of neutral alumina (40 g, Brockmann Grade III). Traces of lycopene were removed with 20% *B/P*, and a second fraction eluted with 10% (v/v) ether in petroleum ether (*E/P*).

This fraction was chromatographed on thin layers of silica gel G, with 10% *E/P* as developing solvent. This gave several bands, two of which were removed and eluted. Band 1 (R_f 0·7) was pale yellow and showed strong greenish fluorescence under u.v. light and band 2 (R_f 0·5) was red. Band 1 was chromatographed on thin layers of MgO : kieselgur G (1 : 1 w/w) with 5% (v/v) acetone in petroleum ether (*A/P*) as developing solvent. Four spots were obtained. Compound *A* (R_f 0·8) was revealed under u.v. light after spraying with a 1% solution of Rhodamine 6G in acetone. *A* had absorption maxima at 275, 285, 296 nm. Compound *B* (R_f 0·6) showed strong greenish fluorescence under u.v. light and had λ_{max} (petroleum ether) at 331, 348, 367 nm. Compound *C* (R_f 0·3) had λ_{max} (petroleum ether) at 404, 426, 451 nm (mutatochrome). Compound *D* (R_f 0·15) had λ_{max} (light petroleum) at 377, 400, 425 nm. Band 2 was rechromatographed on thin layers of MgO : kieselgur G (1 : 1 w/w) with *A/B/P* (2 : 2 : 1) as developing solvent. The major band, compound *E* (R_f 0·4), had λ_{max} (light petroleum) at 445, 471, 503 nm.

Compound *A* was further purified by TLC on silica gel G with 5% *E/P* as developing solvent.

Reactions of Compound A

Attempted acetylation, saponification and $NaBH_4$ reduction were carried out by standard procedures.[4] In each case only starting material was recovered.

Mass Spectrometry

Mass spectrum was determined by I.C.I. (Pharmaceuticals) Ltd., Alderley Edge, through the kindness of Dr. G. A. Snow and Dr. B. Webster. An A.E.I. MS9 instrument was used, with the direct inlet system, and an ion source temperature of 280°.

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[4] G. BRITTON and T. W. GOODWIN, *Methods in Enzymology*, in press.