The conversion of *cis*-ζ-carotene to *trans*-ζ-carotene, proneurosporene, prolycopene, neurosporene, lycopene and γ- and β-carotene by a soluble enzymic system from tangerine tomatoes [9] shows possible conversions which might occur.

EXPERIMENTAL

Pigment extraction and purification. Fresh Tangerine tomato fruits were macerated with Me_2CO–light petrol mixture (bp 30–50°) (1:1) under N_2. The Me_2CO was quickly washed out and the upper layer saponified with 20% KOH in MeOH in the dark under N_2 at room temp. overnight. The extract was transferred to light petrol, washed with H_2O, dried (Na_2SO_4) and concentrated *in vacuo*. Carotenes were chromatographed on Sea Sorb 43 Hyflo–SuperCel (1:2, w/w) with increasing concentrations of Me_2CO in light petrol (up to 15%). The eluate containing the pink band was rechromatographed on the same adsorbent using 1, 2, 4 and 6% Me_2CO in light petrol, the band separating into 2 components. The upper band was γ-carotene and the lower poly-*cis*-γ-carotene. Pigments were eluted from the extruded adsorbent with Me_2CO–light petrol, evaporated to dryness, rechromatographed on alumina II with increasing concentrations of Et_2O in light petrol, and on TLC precoated aluminum oxide (Type E) F_{254} plates with 2% Et_2O in light petrol. Poly-*cis*-γ-carotene was finally purified on alumina II with 20% C_6H_6 and 4% Et_2O in light petrol and TLC on precoated Si gel F_{254} plates as described. Gamma carotene was identified by its absorption spectrum, and by comparison with authentic samples of γ-carotene. Poly-*cis*-γ-carotene was identified by its position on the column below γ-carotene, its absorption spectrum before and after I_2 catalysis and comparison to the literature [4–6].

Absorption spectra. Absorption spectra of carotenes were reported from solns in light petrol (bp 30–50°). Quantitative estimation of poly-*cis*-γ-carotene was made from the equilibrium mixture of stereoisomers after the poly-*cis* pigment was subject to I_2 catalysis using the $E^{1\%}_{1cm}$ value of 3100 for the all-*trans* γ-carotene at 462 nm [10].

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REFERENCES

1. LeRosen, A. L. and Zechmeister, L. (1942) *J. Am. Chem. Soc.* **64,** 1075.
2. Williams, R. J. H., Britton, G., Charlton, J. M. and Goodwin, T. W. (1967) *Biochem. J.* **104,** 767.
3. Raymundo, L. C. and Simpson, K. L. (1972) *Phytochemistry* **11,** 397.
4. Zechmeister, L. (1962) Cis–Trans *Isomeric Carotenoids, Vitamins A and Arylpolyenes*, Springer-Verlag, Vienna.
5. Zechmeister, L. and Schroeder, W. A. (1942) *J. Biol. Chem.* **144,** 315.
6. Zechmeister, L., Rosen, A. L., Schroeder, W. A., Polgar, A. and Pauling, L. (1943) *J. Am. Chem. Soc.* **65,** 1940.
7. Rüegg, R., Schweiter, U., Ryser, G. Schudel, P. and Isler, O. (1961) *Helv. Chem. Acta* **44,** 985.
8. Porter, J. W. and Anderson, D. G. (1962) *Arch. Biochem. Biophys.* **97,** 520.
9. Qureshi, A. A., Qureshi, N., Kim, M. and Porter, J. W. (1974) *Arch. Biochem. Biophys.* **162,** 117.
10. Davies, B. H. (1965) In *Chemistry and Biochemistry of Plant Pigments* (Ed. by T. W. Goodwin), p. 489, Academic Press, New York.

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BACTERIOSTATIC ACTIVITY OF SOME COUMARIN DERIVATIVES

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Key Word Index—Bacteriostatic activity; hydroxycoumarins; furanocoumarins and -glucosides.

Abstract—The bacteriostatic effect of some mono- and dihydroxy-coumarins and isopropyldihydrofuranocoumarins have been examined and compared with other simple phenols. Most of the coumarins proved to be far more bacteriostatic than the simple phenols.

Antimicrobial properties of coumarins are of interest in connection with the possible activity of these compounds as phytoalexins [1–3]. Since vaginidiol and its derivatives are often present in umbelliferous plants we were interested in the bacteriostatic activity of these compounds and specially of apterin [4, 5] and its hydrolysis products, vaginidiol, oroselol and oroselon. Two other derivatives were not investigated: oroselolglucosid because we could not detect it *in vivo* or *in vitro*, and the dimer of oroselon [6, 7] because its formation in a dilute, weakly acidic aqueous solution is very slow. In this paper a comparison of the bacteriostatic activity of some coumarin derivatives with some common bacteriostatic phenols is reported (Table 1). The furanocoumarins proved to be more bacteriostatic than the phenols and also showed more activity than that recorded by other workers [2].

EXPERIMENTAL

Laboratory strains of *E. coli* and *B. subtilis* were used. Overnight cultures of the bacteria on nutrient agar (Difco) were washed once in sterile peptone-saline soln and the cells were resuspended in peptone-saline. Suitable dilutions were made.

Table 1. Bacteriostatic activity of coumarins and phenols

Compound	Concentration in ppm	% Colonies *E. coli*	% Colonies *B. subtilis*
Umbelliferone	65	0	0
Esculetin	65	40	—
	130	—	1
	260	—	0
Daphnetin	33	60	70
	65	0	—
	130	—	0
5,7-dihydroxycoumarin	40	0	0
Esculin	45	—	0
	180	90	—
Apterin	45	0	0
Vaginidiol	65	100	—
Oroselol	130	100	100
Oroselon	saturated	100	50
Phenol	10000	100	100
	40000	10	10
Catechol	500	—	100
	1000	—	70
	2500	35	—
	10000	—	0
	20000	1	—
Resorcinol	10000	100	—
	40000	20	30

Bacterial suspensions containing about 100 and 200 cells were pipetted into sterile plastic petri dishes. 15 ml molten and cooled nutrient agar was poured into the dishes, followed by graded vol of solutions (ethanol or acetone) of the compounds to be investigated. The soln of vaginidiol in alcohol had to be filtered through a G5 filter; it contained a high number of bacteria, most probably due to its preparation by enzymic hydrolysis of apterin. Fluids were mixed by gentle shaking and the gel was allowed to settle. Final readings (number of colonies visible) were made after 48 hr of incubation at 37°. All relevant blanks were run simultaneously. In every instance, the small amount of organic solvent (~2%) present appeared to have a negligible influence (other solvents tested were not innocuous). The bacteriostatic effect is expressed as the percentage of colonies visible vs the blank. (Table 1) Except for catechol, concentrations showing intermediate activities are not presented.

REFERENCES

1. Johnson, C. and Brannon, D. R. (1973) *Phytochemistry* **12,** 2961.
2. Jurd, L., King, A. D., Mihara, K., Corse, J. and Bayn H. (1971) *Phytochemistry* **10,** 2965, 2971.
3. Dadak, V. and Hodak, K. (1966) *Experientia* **XXII,** 38.
4. Steck, W. and Wetter, L. R. (1974) *Phytochemistry* **13,** 1925.
5. Fischer, F. C. and Baerheim Svendsen, A. (1976) *Phytochemistry* **15,** In press.
6. Kamat, V. S., Audichya, T. D., Trivedi, G. K. and Bhattacharyya, S. C. (1975) *J. Chem. Soc. Perkin I*, 204.
7. Fischer, F. C. and Lim, M. I. Unpublished results.

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APTERIN, A COMMON FURANOCOUMARIN GLYCOSIDE IN THE UMBELLIFERAE

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Key Word Index—Umbelliferae: apterin; 8-(2-glucosyloxy)isopropyl-9-hydroxy-8,9-dihydroangelicin.

Apterin (8-(glucosyloxy)isopropyl-9-hydroxy-8,9-dihydroangelicin) has been isolated from *Heracleum mantegazzianum* Somm, et Lev. [1] and from *Zizia aptera* [2] (both Umbelliferae). We have now found it in nine other species of this family. It is present in the roots of *Heracleum sphondylium* L., *H. laciniatum*, *H. sosnosvskyi*, *Pastinaca sativa* L., *Anthriscus silvestris* Pers. (L.) Hoffm. and *Peucedanum palustre* (L.) Moench. It is possibly present in small amounts in *Aegopodium podagraria* L., *Angelica archangelica* L. and *Levisticum officinale* (Hill) Koch, but probably not in *Myrrhis odorata* (L.) Scop., *Petroselinum crispum* (Miller) Hill and *Pimpinella saxifraga* L. However, the amount of apterin present in the roots of *H. mantegazzianum* seems to depend upon the stage of development and the growing conditions of the plant [3]. Such variations may also occur in other species, so we cannot conclude that it is necessarily absent from the three species mentioned above. The aglucone, vaginidiol, has not as yet been found in any of the plants at our disposal.

EXPERIMENTAL

Plant sources. Plants of known taxonomic authenticity were put at our disposal by the Hortus Botanicus of the University of Leyden and by the municipal "Heempark" of Leyden.

Methods. The screening method used was a small-scale version of our original isolation procedure for apterin [1]. At least three different TLC systems were used to examine the isopropanol extract for the presence of apterin; when enough apterin was present, it was purified by PLC and its identity confirmed by following (UV-spectroscopy) its acid hydrolysis (apterin → oroselol + oroselon → dimers [4]. Steck and Wetter [2] reported unsuccessful attempts to hydrolyse apterin by means of emulsin. We obtained a complete conversion with emulsin in H_2O–MeOH for 3 months. The suggestion made by Steck and Wetter about the steric hindrance protecting the glucosidic bond is supported by Röntgen-diffraction data [5].