Triterpenoid Carotenoids and Related Lipids

TRITERPENOID CAROTENOID ALDEHYDES FROM *STREPTOCOCCUS FAECIUM* UNH 564P

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1. The identification of two novel triterpenoid xanthophylls from *Streptococcus faecium* UNH 564P is described. 2. Both are aldehydes and were identified as 4,4'-diaponeurosporen-4-al and 4,4'-diapolycopen-4-al. 3. A pathway is presented for the biosynthesis of these and other triterpenoid carotenoids in *S. faecium*.

We reported the occurrence in nature of the first exclusively triterpenoid (C₃₀) carotenoid series, in the non-photosynthetic Gram-positive bacterium *Streptococcus faecium* UNH 564P (Taylor & Davies, 1973; 1974*a,b*; see Scheme 1 for details). The carotene series (Taylor & Davies, 1974*a*) proceeds from 4,4'-diapophytoene (I; 4,4'-diapo-7,8,11,12,7',8',11',12'-octahydro- ψ,ψ -carotene), by dehydrogenation through 4,4'-diapophytofluene (II; 4,4'-diapo-7,8,11,12,7',8'-hexahydro- ψ,ψ -carotene) and the isomeric conjugated heptaenes, 4,4'-diapo-7,8,11,12-tetrahydrolycopene (III; 4,4'-diapo-7,8,11,12-tetrahydro- ψ,ψ -carotene) and 4,4'-diapo- ζ -carotene (V; 4,4'-diapo-7,8,7',8'-tetrahydro- ψ,ψ -carotene), to terminate in 4,4'-diaponeurosporene (VII; 4,4'-diapo-7,8-dihydro- ψ,ψ -carotene). The latter was isolated in its all-*trans* (VII), neo B (VI) and neo C (IV) forms. 4,4'-Diaponeurosporene appears to act as the substrate for xanthophyll formation since the two major xanthophylls present (Taylor & Davies, 1974*b*), 4-hydroxy-4,4'-diaponeurosporene (X; 4,4'-diapo-7',8'-dihydro- ψ,ψ -caroten-4-ol) and its glucoside (XI; 4-D-glucopyranosyloxy-4,4'-diapo-7',8'-dihydro- ψ,ψ -carotene), both retain the 4,4'-diaponeurosporene chromophore (Scheme 1). Taylor & Davies (1974*b*) also described two further xanthophylls as 'carbonyl-containing 4,4'-diapocarotenoids'. The present paper is on the identification of these as aldehydic C₃₀ apocarotenoids and suggests how both may be derived from 4-hydroxy-4,4'-diaponeurosporene by further oxidation.

Materials and Methods

Methods for the growth, harvesting and determination of bacterial dry wt. of *Streptococcus faecium* UNH 564P, for the extraction, manipulation and spectrophotometric determination of carotenoids and the source and purification of solvents were as described by Taylor & Davies (1974*a,b*).

Purification of carotenoids

The lipid fraction from harvested cells of *S. faecium* was partitioned between light petroleum (b.p. 40–60°C) and aq. 95% (v/v) methanol (Taylor & Davies, 1974*a*). Chromatography of the epiphasic fraction on a column (24 cm × 2 cm) of alumina (M. Woelm, Eschwege, Germany; neutral, Brockmann activity grade II), packed in light petroleum and developed with light petroleum followed by increasing concentrations of acetone in light petroleum, led to the resolution of ten carotenoid fractions. The components of fractions I–VII, eluted successively with 0–2% (v/v) acetone in light petroleum, have been identified as 4,4'-diapocarotenes (Taylor & Davies, 1974*a*; see Scheme 1 for structures) and the identification of the carotenoids of fractions VIII and IX, which were eluted with 4 and 7.5% (v/v) acetone in light petroleum respectively, is reported below. Fraction X, eluted with 15–20% (v/v) acetone in light petroleum, has already been identified as 4-hydroxy-4,4'-diaponeurosporene (Taylor & Davies, 1974*b*).

Fractions VIII and IX were further purified by preparative t.l.c. (see below) followed by repeated chromatography on columns (15 cm × 1 cm) of alumina (neutral, grade II) developed, as appropriate, with either 4 or 8% (v/v) acetone in light petroleum. The chromatographically pure carotenoids (VIII and IX) had absorption maxima in light petroleum at 444 (infl.), 466 and 496 nm and at 454 (infl.), 476 and 506 nm respectively.

Chemical tests

Procedures for acetylation and silylation of carotenoids and for the dehydration (with HCl-saturated chloroform) of carotenoids containing allylic hydroxyl groups have been described (Taylor & Davies, 1974*b*).

Reduction of carbonyl groups (Krinsky &

Goldsmith, 1960) was accomplished by treating an ethanolic solution of the carotenoid with a few crystals of NaBH_4 . The resulting solution was kept in the dark under N_2 at 4°C for 12–18 h. The reduction product could be recovered by the addition of 2 vol. of water and extraction of the carotenoid with diethyl ether.

The properties of the products of each of the above tests were compared with those of the starting materials or of appropriate authentic compounds by t.l.c., electronic-absorption spectrophotometry and the determination of partition coefficients. The latter were determined between light petroleum (b.p. $40\text{--}60^\circ\text{C}$) and aq. 95% (v/v) methanol by the method of Petracek & Zechmeister (1956) and expressed as the ratio of the percentages of the total carotenoid in each of the two phases (epiphase/hypophase) as determined by the extinctions at the wavelength of maximal absorbance.

Thin-layer chromatography

T.l.c. was on thin layers (0.25 mm) of silica gel G (E. Merck, Darmstadt, Germany) in closed glass tanks that were lined with chromatography paper (Whatman no. 1; Whatman Biochemicals, Maidstone, Kent, U.K.) and pre-saturated with solvent for at least 1 h before use. The four solvent systems used (Tables 1 and 2) were: 1, benzene/methanol/acetic acid (87:11:2, by vol.); 2, chloroform; 3, 10% (v/v) acetone in light petroleum (b.p. $40\text{--}60^\circ\text{C}$); 4, 15% (v/v) acetone in light petroleum. Carotenoids were detected on t.l.c. plates by their colour and as brown spots on exposure to I_2 vapour.

Preparative t.l.c. used in the purification of fractions VIII and IX was on 0.5 mm layers of silica gel G in either solvent 3 (for VIII) or 4 (for IX). The purified carotenoids were recovered by scraping the appropriate zones from the plates and eluting the adsorbent with diethyl ether.

Partial synthesis of 4,4'-diapolycopene (4,4'-diapo- ψ,ψ -carotene)

Squalene may be dehydrogenated by *N*-bromo-succinimide to yield a series of coloured products (Dale, 1952); the most unsaturated of these is a conjugated undecaene, the C_{30} analogue of lycopene (4,4'-diapolycopene). A sample of this fully conjugated triterpenoid carotene was prepared by an adaptation of the method of Zechmeister & Koe (1954).

Squalene (500 mg; Eastman Kodak, Kirkby, Lancs., U.K.) was dissolved in carbon tetrachloride (37.5 ml), and acetic acid (12.5 ml) and *N*-bromo-succinimide [132 mg; Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K.] were added. The solution was refluxed for 15–20 min until it was

orange-brown and then cooled in ice and neutralized by washing with 100 ml of aq. 5% (w/v) NaHCO_3 solution. The mixture was washed with 3×100 ml of water and then with 2×100 ml of saturated NaCl solution. Concentration to dryness *in vacuo* yielded a residue that was chromatographed on a column of alumina (Woelm neutral, grade II) with increasing concentrations (0–4%, v/v) of acetone in light petroleum. The most polar of a large number of coloured zones (eluted with 4% acetone), which also had the longest wavelength maxima and was presumably the all-*trans* isomer of the final desaturation product, was collected and repurified by t.l.c. by using, successively, silica gel G developed with 15% (v/v) benzene in *n*-hexane and then silica gel G developed with 10% (v/v) acetone in hexane. The all-*trans* 4,4'-diapolycopene (yield less than 1%) had λ_{max} in light petroleum at 414 (infl.), 440, 466, and 498 nm.

Mass spectra

Mass spectra of purified carotenoids were determined on an A.E.I. MS 30 instrument by a direct-insertion probe sampling technique. The probe temperature was $100\text{--}175^\circ\text{C}$, and ionization potentials of 18 or 24 eV were used.

Results

Identification of carotenoid (VIII) as 4,4'-diaponeurosporen-4-al

The positions of the absorption maxima of the carotenoid (VIII) in light petroleum, at 444 (infl.), 466 and 496 nm, indicated a chromophore of more than 9 conjugated double bonds (Davies, 1976). The absorption spectra in ethanol or chloroform, however, each had only one maximum (at 468 and 470 nm respectively), the loss of persistence in ethanol indicating the conjugation of a carbonyl group with the polyene chain (Weedon, 1969). These spectral data are consistent with the carotenoid (VIII) having a conjugated nonaene chromophore extended by conjugation with a carbonyl group.

The chromatographic mobility of the carotenoid [eluted from grade II alumina with 4% (v/v) acetone in light petroleum] was also consistent with the presence of a carbonyl oxygen (Davies, 1976). The carotenoid (VIII) was eluted after the most polar of the hydrocarbon carotenes (4,4'-diaponeurosporene; VII; 2% acetone in light petroleum) but before the monohydroxycarotene (X; 15–20% acetone in light petroleum).

The failure of the carotenoid (VIII) either to acetylate or to silylate confirmed the absence of hydroxyl functions, and the presence of a conjugated carbonyl group was verified by the reaction of the compound with NaBH_4 in ethanol. Its absorption

Table 1. *Spectroscopic, partition and adsorption characteristics of the epiphasic carotenoid (VIII) and its reaction products, and a comparison with the properties of authentic 4-hydroxy-4,4'-diaponeurosporene*

For details see the text.

Sample	Absorption maxima (nm) in the given solvent	Partition coefficient in light petroleum/95% methanol	<i>R_F</i> values on t.l.c. in system:			
			1	2	3	4
Carotenoid (VIII)	(444) 466 496 Light petroleum 468 Ethanol 470 Chloroform	56:44	0.72	0.70	0.36	0.59
Products obtained by:						
(a) reduction with NaBH ₄ (386)	413 435 465 Light petroleum 415 438 467 Ethanol 423 449 477 Chloroform	25:75	0.66	0.33	0.13	0.26
(b) reduction followed by treatment with HCl/chloroform (397)	425 447 477 Light petroleum 428 450 480 Ethanol 439 460 489 Chloroform	87:13	0.74	0.60	0.50	0.67
4-Hydroxy-4,4'-diaponeurosporene (X)	(386) 413 435 465 Light petroleum 415 438 467 Ethanol 423 449 477 Chloroform	26:74	0.67	0.31	0.12	0.25
4-Hydroxy-4,4'-diaponeurosporene treated with HCl/chloroform (397)	424 447 477 Light petroleum 427 450 479 Ethanol 438 460 489 Chloroform	89:11	0.75	0.61	0.51	0.67

maximum underwent a hypsochromic shift of 30 nm and the absorption spectrum of the product showed the fine structure and three persistent maxima at wavelengths characteristic of an acyclic conjugated nonaene chromophore (Table 1). Comparisons of this reduction product with authentic 4-hydroxy-4,4'-diaponeurosporene (X) from *S. faecium* showed the two compounds to be identical in their partition coefficient, in their absorption maxima in three solvents, in their chromatographic mobility in four t.l.c. systems and in their reaction with HCl-saturated chloroform (Table 1). The latter test, in which a carotenoid containing a hydroxyl group allylic to a conjugated nonaene would be dehydrated to yield a conjugated decaene with a *retro* structure (Liaaen-Jensen, 1971), resulted in the formation of products that were identical in their partition coefficients, absorption spectra and chromatographic behaviour (Table 1). It is clear from the chemical tests that the carotenoid (VIII) is the aldehyde that reduces to yield 4-hydroxy-4,4'-diaponeurosporene (X), and must therefore be formulated as 4,4'-diaponeurosporen-4-al (Scheme 1).

This identification was amply confirmed by the mass-spectral data. The mass of the molecular ion at *m/e* 416 (41%) corresponded to that calculated for C₃₀H₄₀O, and ions at *m/e* 398 (20%; *M*-18), 388 (29%; *M*-28) and 387 (12%; *M*-29) indicated the presence of an aldehydic carbonyl group conjugated with the polyene chain (Enzell, 1969). Ions at *m/e* 347 (4%; *M*-69) and 69 (base peak) arose from 'bis-allylic' fission of the saturated 7',8'-bond, whereas cleavage of the 8',9'-bond led to ions at *m/e* 333 (8%; *M*-83) and

83 (86%); both types of fragmentation are known to be characteristic of 4,4'-diaponeurosporene (VII; Taylor & Davies, 1974a), 4-hydroxy-4,4'-diaponeurosporene (X) and 4-D-glucopyranosyloxy-4,4'-diaponeurosporene (XI; Taylor & Davies, 1974b). Characteristic carotenoid rearrangement losses of toluene (92 mass units) and *m*-xylene (106 mass units) were observed in ions at *m/e* 324 (2%; *M*-92) and 310 (6%; *M*-106) and combination losses led to the appearance of ions at *m/e* 319 (35%; *M*-28-69), 305 (18%; *M*-28-83), 296 (16%; *M*-28-92) and 282 (29%; *M*-28-106).

All the analytical data are consistent with the carotenoid (VIII) being formulated as 4,4'-diaponeurosporen-4-al (4,4'-diapo-7',8'-dihydro- ψ,ψ -caroten-4-al) or 2,6,10,15,19,23-hexamethyltetracos-2,4,6,8,10,12,14,16,18,22-decaen-1-al.

Identification of carotenoid (IX) as 4,4'-diapolycopene-4-al

The longest-wavelength absorption maxima of all the carotenoids of *S. faecium* were those of carotenoid (IX), which had absorption bands in light petroleum at 454 (infl.), 476 and 506 nm. As had been the case with 4,4'-diaponeurosporen-4-al (VIII), the fine structure was lost when the absorption spectrum was determined in ethanol or chloroform, single maxima at 477 or 488 nm respectively being recorded. These data suggest that the carotenoid (IX) has a conjugated undecaene chromophore [cf. 4,4'-diapolycopene, $\lambda_{\max}$ 414 (infl.), 440, 466 and 498 nm in light petroleum] extended by further conjugation with a

carbonyl group. Such a structure differs from that of 4,4'-diaponeurosporen-4-al (VIII) in that it contains an additional (7',8') olefinic bond, which should slightly increase its adsorption affinity for alumina. The carotenoid (IX) was eluted from columns of alumina (neutral, grade II) with 7.5% (v/v) acetone in light petroleum and was therefore slightly more polar than 4,4'-diaponeurosporen-4-al (VIII; 4% acetone in light petroleum) but much less polar than 4-hydroxy-4,4'-diaponeurosporene (X; 15–20% acetone in light petroleum). Comparisons of its spectroscopic and chromatographic properties with those of 4,4'-diaponeurosporen-4-al (VIII) permit the formulation of the carotenoid (IX) as 4,4'-diapolycopene-4-al.

The mass spectrum was fully consistent with this structure. The molecular ion at m/e 414 (19%) corresponded to the postulated molecular formula of $C_{30}H_{38}O$. Ions at m/e 396 (14%; $M-18$), 386 (58%; $M-28$) and 385 (6%; $M-29$) indicated the presence of an aldehydic carbonyl group conjugated with the polyene chain (Enzell, 1969). That the major fission of the polyene chain occurred between carbons 8' and 9' was indicated by the base peak at m/e 81 and a combination loss yielding an ion at m/e 305 (27%; $M-28-81$). The presence in the spectrum of these ions, and the absence of any significant ions corresponding to $M-69$ and $M-83$ (cf. 4,4'-diaponeurosporen-4-al, see above), indicated that the polyene chain extended throughout the molecule. Other combination losses, corresponding to carotenoid rearrangement losses of toluene and m -xylene from the polyene chain coupled with the loss of 28 mass units from the conjugated aldehyde function and (in some cases) with the fission of the 8',9'-single bond, led to the appearance of ions at m/e 294 (3%; $M-28-92$), 280 (9%; $M-28-106$), 213 (22%; $M-28-81-92$) and 199 (35%; $M-28-81-106$).

All these data are consistent with the formulation of the carotenoid (IX) as 4,4'-diapolycopene-4-al

(4,4'-diapo- ψ,ψ -caroten-4-al) or 2,6,10,15,19,23-hexamethyltetracos-2,4,6,8,10,12,14,16,18,20,22-undecaen-1-al.

A number of simple chemical tests were performed on the carotenoid. The absence of hydroxyl groups was confirmed by its failure to undergo acetylation or silylation. The presence of a (non-carboxylic) carbonyl function conjugated with the polyene chain was confirmed by the reaction of the compound with $NaBH_4$ in ethanol. There was a marked hypsochromic shift (23 nm) and the absorption spectrum of the product showed the fine structure and persistent maxima characteristic of an acyclic conjugated polyene chromophore (Table 2). The expected reduction product of 4,4'-diapolycopene-4-al (IX) would be the corresponding primary alcohol, 4-hydroxy-4,4'-diapolycopene (4,4'-diapo- ψ,ψ -caroten-4-ol), a compound with a conjugated undecaene chromophore. The actual product (Table 2), however, had maxima in light petroleum at 404 (infl.), 426, 452 and 482 nm, corresponding more to a conjugated decaene chromophore, as in spheroidene [1-methoxy-1,2,7',8'-tetrahydro-3,4-didehydro- ψ,ψ -carotene; λ_{max} . 405 (infl.), 427, 453 and 484 nm in light petroleum (Davies, 1970b)]. Possible reasons for this anomaly are discussed below. The reduction product of the carotenoid (IX) was slightly more polar than that of 4,4'-diaponeurosporen-4-al (i.e. 4-hydroxy-4,4'-diaponeurosporene) in the partition test and in four t.l.c. systems (Table 2); such a difference in polarity is consistent with the postulated difference (of one olefinic bond) between the structures. Unlike that of 4,4'-diaponeurosporen-4-al (cf. Table 1), the $NaBH_4$ reduction product of carotenoid (IX) did not give the expected reaction with HCl-saturated chloroform. This reaction, commonly used as a test for allylic hydroxyl groups (Liaaen-Jensen, 1971), gave an atypical product which had a weak and multi-peaked absorption spectrum and which was less polar than anticipated on t.l.c. (cf. Tables 1 and 2).

Table 2. Spectroscopic, partition and adsorption characteristics of the epiphasic carotenoid (IX) and of its reaction products

For details see the text.

Sample	Absorption maxima (nm) in the given solvent	Partition coefficient in light petroleum/95% methanol	R _F values on t.l.c. in system:			
			1	2	3	4
Carotenoid (IX)	(454) 476 506 Light petroleum 477 Ethanol 488 Chloroform	49:51	0.72	0.70	0.30	0.52
Product obtained by:						
(a) reduction with $NaBH_4$	(404) 426 452 482 Light petroleum (405) 428 454 484 Ethanol (410) 438 464 496 Chloroform	19:81	0.59	0.25	0.10	0.18
(b) reduction followed by treatment with HCl/chloroform	Poorly-defined and multi-peaked spectrum in all three solvents	—	0.76	0.79	0.71	0.81

Quantitative determination of the carotenoids (VIII and IX)

The two carotenoids (VIII and IX) are but minor constituents of the total pigment fraction of *S. faecium*. They were estimated spectrophotometrically by assuming that their specific extinction coefficients ($E_{1\text{cm}}^{1\%}$) in light petroleum are 3905 [i.e. that calculated for 4,4'-diaponeurosporene (Taylor & Davies, 1974a)], at their respective absorption maxima. When extracted from cells cultured under the standard conditions, 4,4'-diaponeurosporen-4-al represented 0.31% (0.081 $\mu\text{g/g}$ cell dry wt.; 0.015 mg/g of ether-extractable lipid) and 4,4'-diapolycopene-4-al, 0.47% of the total carotenoid (0.12 $\mu\text{g/g}$ cell dry wt.; 0.022 mg/g of lipid) of *S. faecium*. This meant that some 2.8 μg of the former and 4.2 μg of the latter were obtained from each 45-litre batch culture. The studies described above necessitated the growing of some 450 litres of the organism.

Discussion

The evidence for the structure of carotenoid (VIII) as 4,4'-diaponeurosporen-4-al is strong. Its spectroscopic characteristics, adsorption behaviour and mass spectrum are all consistent with this formulation, which is amply confirmed by reduction of the carotenal with NaBH_4 to yield 4-hydroxy-4,4'-diaponeurosporene (X). The identification of the other carotenoid (IX) as 4,4'-diapolycopene-4-al is a little more tentative. Although its physical properties, including its mass spectrum, and its reduction with NaBH_4 fully support the proposed structure, certain difficulties were experienced in investigating the nature of the reduction product.

The hypsochromic shift occurring on reduction (in ethanol) of 4,4'-diaponeurosporen-4-al (VIII) was 30 nm (Table 1) and that for 4,4'-diapolycopene-4-al (IX) was 23 nm (Table 2); both shifts are of the order expected for the reduction of an aldehydic carbonyl group conjugated with an acyclic polyene chain (Davies, 1976). Whereas the reduction product of

(VIII) had absorption maxima in ethanol (415, 438 and 469 nm) corresponding to those of a conjugated nonaene chromophore, the other carotenal (IX) reduced to yield a compound with maxima at 405 (infl.), 428, 454 and 484 nm (ethanol), that is at values corresponding more to a conjugated decaene (see above) than to the expected undecaene chromophore [e.g. lycopene, λ_{max} in ethanol at 446, 472 and 503 nm (Davies, 1976)].

One possible explanation that may be advanced to account for this apparent discrepancy is that because the conjugated undecaene chromophore in a triterpenoid carotenoid runs the entire length of the molecule, its absorption maxima may be at shorter wavelengths than those of a similar chromophore in a C_{40} carotenoid, in which the conjugated undecaene is flanked by isolated olefinic bonds (cf. structures of lycopene and 4,4'-diapolycopene below). That such isolated double bonds do influence the absorption maxima of chromophores has been demonstrated for conjugated trienes (Khatoon *et al.*, 1972). This possibility was examined by carrying out a partial synthesis from squalene of 4,4'-diapolycopene, the C_{30} analogue of lycopene; it had absorption maxima in light petroleum at 414 (infl.), 440, 466 and 498 nm, i.e. at wavelengths some 6–7 nm lower than those of C_{40} lycopene [414 (infl.), 446, 473 and 505 nm in light petroleum (Davies, 1970a)]. Similarly, 4,4'-diaponeurosporene (VII) has its maxima at wavelengths some 2–4 nm less than those of C_{40} neurosporene [7,8-dihydro- ψ,ψ -carotene (Davies, 1970a; Taylor & Davies, 1974a)]. The above suggestion is therefore valid, but the effect is not of a sufficient magnitude to explain the discrepancy.

Again from studies of C_{40} carotenoids, it is known that successive desaturations of acyclic carotenes result in bathochromic shifts of decreasing magnitude. Thus neurosporene, lycopene, 3,4-dehydrolycopene (3,4-didehydro- ψ,ψ -carotene) and bisdehydrolycopene (3,4,3',4'-tetrahydro- ψ,ψ -carotene), with chromophores of 9, 11, 13 and 15 conjugated double bonds, have their main (central) absorption maxima in light petroleum at 439, 472, 492 and 510 nm

4,4'-Diapolycopene (C_{30})

Lycopene (C_{40})

Scheme 1. Pathway of triterpenoid carotenoid formation in *S. faecium* UNH 564P

respectively (Davies, 1976). The successive bathochromic shifts decrease from 33 to 20 and then to 18 nm as the molecule becomes more unsaturated and, finally, fully conjugated. The difference of 17 nm (see Tables 1 and 2) between the main absorption maxima (in light petroleum) of the reduction products of the carotenals (VIII and IX) is of the same order (as the final 18 nm shift) and would be consistent with completing the conjugation of the C₃₀ molecule.

A further explanation of the inconsistencies in the properties of the reduction product of (IX) might be that the conditions used for NaBH₄ reduction result in chemical changes other than, or in addition to, those expected. This may be borne out by the failure of the product, unlike that of the other carotenal (VIII), to yield a product with well-defined properties on treatment with HCl-saturated chloroform in the test for an allylic hydroxyl group.

Notwithstanding the ambiguity in its behaviour under the conditions of NaBH₄ reduction, the more polar carotenal (IX) had all other properties consistent with its formulation as 4,4'-diapolycopene-4-al. Further structural investigations were not possible on the pooled 40 µg sample obtained from 450 litres of culture and the preparation of further samples was precluded by expense.

The characterization of the two triterpenoid carotenals completes our studies on the nature of the carotenoids of *S. faecium* UNH 564P. The biosynthetic pathway formulated as a result of all these studies is shown in Scheme 1.

The dehydrogenation sequence of the carotenes terminates in 4,4'-diaponeurosporene (VII; Taylor & Davies, 1974a), which is then converted into its primary alcohol, 4-hydroxy-4,4'-diaponeurosporene (X; Taylor & Davies, 1974b). The oxidation of the terminal methyl group presumably takes place in a manner analogous to that of the ω -hydroxylation of *n*-alkanes by enzymes from *Corynebacterium* 7E1C (flavoprotein, non-haem iron, cytochrome P-450) and *Pseudomonas oleovorans* (rubredoxin; Jurtshuk & Cardini, 1972). This appears to be the only route for the metabolism of 4,4'-diaponeurosporene, since no 4,4'-diapolycopene has been detected in *S. faecium* (Taylor & Davies, 1974a).

Under normal culture conditions, the 4-hydroxy-4,4'-diaponeurosporene is converted into 4-D-glucopyranosyloxy-4,4'-diaponeurosporene (XI; Taylor & Davies, 1974b), but the present studies

indicate that further oxidation of the primary alcohol may also occur, to yield 4,4'-diaponeurosporen-4-al (VIII) as a minor component. The formation of the glucoside is influenced, of course, by the medium glucose concentration (Taylor & Davies, 1976); if this is low, or if glucose is absent, the formation of aldehydes becomes the major metabolic pathway (B. H. Davies & R. F. Taylor, unpublished work).

As a final metabolic step, 4,4'-diaponeurosporen-4-al (VIII) is dehydrogenated to 4,4'-diapolycopene-4-al (IX) by a reaction that completes the desaturation of the molecule. That 4,4'-diapolycopene-4-al is formed specifically by this route is indicated by the apparent absence from all cultures of both 4,4'-diapolycopene and 4-hydroxy-4,4'-diapolycopene.

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