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CAROTENOIDS OF *ERIOBOTRYA JAPONICA*

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Key Word Index—*Eriobotrya japonica*; Rosaceae; Maloideae; loquat fruit; pulp; peel; cartenoids.

Abstract—The carotenoids of the loquat fruit *Eriobotrya japonica* Golden Nugget variety, were investigated. They were identified according to their chromatographic, spectrophotometric and chemical properties and compared with standard pigments. For some of the carotenoids, MS were determined. Pulp and peels were investigated separately. The main pattern of the pulp carotenoids was β-carotene (33%), γ-carotene (6%), cryptoxanthin (22%), lutein, violaxanthin and neoxanthin, each about 3–4%. The peel, with a carotenoid content 5 times as high, had a similar pattern, but the ratio between the main pigments differed: β-carotene (50%); γ-carotene (5%); cryptoxanthin (5%); lutein (13%); violaxanthin, neoxanthin, 3–4%. The carotenoids of the loquat (subfamily Maloideae) were very similar to those of the apricot (*Prunus armeniaca*–subfamily Prunoideae) both of the family of *Rosaceae*. The intergeneric differences are more pronounced, which is of possible taxonomic significance. The lower concentration of cryptoxanthin and the high concentration of lutein in the peels is noteworthy and of biosynthetic interest.

INTRODUCTION

IN RECENT years the carotenoids of several fruit species grown in Israel have been reinvestigated in our laboratory using new analytical methods.[1–5]

Knowledge of the carotenoids of higher plants is of importance in areas such as chemotaxonomy and carotenogenesis, as well as being of practical importance, e.g. determining the colour of fruit and vegetables.

The carotenoids of the loquat fruit, which are mainly responsible for its colour, have not been previously investigated. The fruit originates in eastern Asia and is one of the exotic (subtropical) fruits that are successfully cultivated in Israel. The plant belongs to the family Rosaceae, subfamily Maloideae. The pear shaped golden orange fruit with an agreeable acid flavour is about 37 mm long and has a thin endocarp. It contains a few large ovoid or angular brown smooth seeds that constitute about 25% of the total fruit weight.

RESULTS AND DISCUSSION

Table 1 represents the qualitative distribution of the carotenoids of the loquat endocarp, including spectrophotometric and chromatographic properties as well as chemical tests.

Two unknown light yellow pigments were noted during the chromatographic separation of the colourless compounds phytoene and phytofluene that did not have the characteristic

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[1] GROSS, J. and LIFSHITZ, A. (1970) *Lebensm.-Wiss. Technol.* **3**, 52.
[2] GROSS, J., GABAI, M. and LIFSHITZ, A. (1971) *J. Food Sci.* **36**, 466.
[3] GROSS, J., GABAI, M. and LIFSHITZ, A. (1972) *Phytochemistry* **11**, 303.
[4] GROSS, J., GABAI, M. and LIFSHITZ, A. (1972) *J. Food Sci.* **37**, 589.
[5] ROTSTEIN, A., GROSS, J. and LIFSHITZ, A. (1972) *Lebensm.-Wiss. Technol.* **5**(4), 140.

TABLE 1. CHARACTERIZATION OF CAROTENOIDS OF THE LOQUAT FRUIT *Eriobotrya*

Identification	Abs. max. (nm)	Eluent from MgO–Hyflo Super Cel, 1:1	Alumina Grade I
Phytoene	271, 283, 297	P	5%E/P
Unknown a	414, 440		
Unknown b	394, 417		
Phytofluene	328, 347, 466		
cis-neo-β-Carotene	335, 420, 443, 473	2–5%A/P	5–10%E/P
β-Carotene	422, 449, 478	5–10%A/P	10–20%E/P
ζ-Carotene	378, 398, 424		
γ-Carotene	440, 465, 495		20–50%E/P
Mutatochrome	400, 424, 448		50%E/P
Carbonyl 450	450		Et
γ-Carotene-like	438, 463, 493	10%A/P	
Cryptoxanthin 5,6,5′,6′-diepoxide	415, 438, 468		
Cryptoxanthin 5,6-epoxide	418, 440, 467		
Cryptoxanthin 5′,6′-epoxide	420, 443, 470		
cis-Cryptoxanthin	330, 420, 445, 473		
Cryptoxanthin	420, 448, 474		
Cryptoxanthin 5,6,5′,8′-diepoxide	396, 420, 444		
Cryptoflavin	400, 423, 448	10%A/P–Et (99:1)	
Lutein	418, 442, 468		
cis-Lutein	328, 416, 438, 465		
Isolutein	415, 437, 464		
Violaxanthin	415, 437, 466		
Chrysanthemaxanthin	400, 422, 447	10%A/P–Et (97:3)	
Auroxanthin	377, 400, 425		
Luteoxanthin	395, 418, 445		
Neochrome	398, 420, 448		
Neoxanthin	415, 438, 468		
Neoxanthin-like	413, 436, 465		

* Components of solvent systems are abbreviated to: P—petrol; A—acetone; Et—ethanol;

three-maxima spectrum typical of carotenoids. These two pigments had two maxima, one with the main peak at 440 nm and the other with the main peak at 417 nm. At times, these two pigments were not resolved by column chromatography, giving a 'normal' carotenoid spectrum with peaks at 394, 417 and 440 nm and were only resolved by TLC. This weakly adsorbed pigment mixture displayed unique spectral and adsorptive properties being similar chromatographically to ε-carotene, found in a tomato strain (delta tangerin).[6] It is also very similar spectrophotometrically to the ε-carotene found in flamingo[7] (maxima 393, 414, 438, 468 nm) but without the maxima at the longer wavelength.

The mass spectrum of the separated component 'unknown *a*' (414, 440 nm) showed *m*/*e* 586 (5%, presumed M^+); 571 (0·4% [M-15]); 557 (0·8% [M-29]); 530 (80% [M-56]);

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[6] WILLIAMS, J. H., BRITTON, G., CHARLTON, M. J. and GOODWIN, T. W. (1967) *Biochem. J.* **104,** 262.

[7] FOX, D. L. and HOPKINS, T. S. (1966) *Compar. Biochem. Physiol.* **17,** 841.

japonica GOLDEN NUGGET VARIETY, INCLUDING CHEMICAL TESTS AND PURIFICATION*

TLC		Epoxide test		Carbonyl test
Absorbent	Solvent system	Colour	Hypso chromic shift (nm)	Reduced pigment Abs. max. (nm)
MgO-Kieselgur G 1:1	P			
	2–5%A/P			
	2%A/P			
Alumina	5%A/P	Blue		39, 417, 442
		Blue	40	
		Green	20	
		Green	20	
Alumina or MgO-Kieselgur	10%A/P			
		Blue	20	
		Blue		
		Green	19	
	20%A/P or P.E.I.		40	
		Green		
Silica gel		Blue	20	
	30%A/P or P.E.I.		20	
		Blue–Green	18	

E—diethylether; P.E.I.—petrol–ethylacetate–isopropanol (19:2:1).

516 (3%); 474 (1%); 473 (1%); 422 (3%); 410 (100%); 408 (15%); 402 (8%); 395 (16%); 393 (15%); 351 (25%); 309 (17%); 281 (55%); 279 (75%). The presumed molecular ion at 586 m.u. (atomic mass units) was not present in later scans, which still revealed a small broadened peak at 480 m.u. This may thus be due to M-106 and/or a metastable decomposition 586 → 530. The loss of 56 m.u. is associated with an α-carotene end group.[8]

In the hydrocarbon group, the predominant pigment was shown to be all-*trans*-β-carotene. It was identical with synthetic β-carotene (Hoffmann La Roche). The MS was closely similar to that of authentic β-carotene with the expected large ratio of (M-92)/(M-106).[9] It was accompanied by a single *cis*-form and by a single oxygenated derivative, mutato-

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[8] VETTER, W., ENGLERT, G., RIGASSI, N. and SCHWIETER, U. (1971) in *Carotenoids* (ISLER, O., ed.), Chap. IV, p. 243, Birkhäuser, Basel.

[9] ENZELL, C. R., FRANCIS, G. W. and LIAAEN-JENSEN, S. (1968) *Acta Chem. Scand.* **22**, 1054.

chrome. The MS of the latter, 552 (90%, M^+); 537 (15% [M-15]); 536 (15% [M-16]); 472 (100% [M-80]); 460 (15% [M-92]); 446 (40% [M-106]); 349 (10%); 336 (18%); 205 (300%); 165 (60%), was in good accord with that reproduced by Budzikiewicz.[10] In particular, M-80 is in accord with the oxide structure[8] and the last two peaks, the largest in their respective groups, are in accord with the absence of a hydroxyl group in the oxidized end-group.[8]

β-Carotene was followed by ζ-carotene, with its characteristic colour and spectrum, and the latter was followed by γ-carotene. Its MS revealed: 536 (100%, M^+); 534 (10% [M-2]); 444 (17% [M-92]); 430 (22% [M-106]); 375 (8% [M-92-69]); 361 (8% [M-106-69]). The spectrum and the ratio (M-92)/(M-106) are in good accord with the data recorded by Schwieter.[11] This pigment was identical with γ-carotene from carrots and tomatoes. It could not be acetylated. An unknown, more polar pigment with absorption maxima like γ-carotene, presented special difficulties in that it could not be rechromatographed on thin-layer plates or acetylated without decomposing. It had the same polarity as lycopene but had a red–violet colour on thin-layer plates.

The carbonyl pigment 450 was accompanied by small quantities of other carbonyl pigments having absorption maxima at shorter wavelengths. Although it has a spectrum similar to β-apo-8′-carotenal, it was more polar on TLC alumina developed with 2–5% acetone in petrol. Its reduction spectrum has the main maximum at 417 nm as compared to maximum at 423 nm of reduced β-apo-8′-carotenal.

The monol fraction contained mainly cryptoxanthin, which was accompanied by nearly all its epoxides. In column chromatography, cryptoxanthin was preceded by its 5,6,5′,6′-diepoxide followed by the two 5,6-monoepoxides. Upon TLC on alumina they separated in inverse order, cryptoxanthin being the less polar. Cryptoxanthin and cryptoflavin were compared and were identical with the natural citrus pigments.

Cryptoxanthin diepoxide showed in the MS: 584 (100%, M^+); 582 (10% [M-2]); 568 (10% [M-16]); 504 (60% [M-80]); 492 (3% [M-92]); 478 (1% [M-106]); 221 (>100%); 205 (>100%); 165 (>100%). Besides the molecular ion, the losses of 16 and 80 m.u. is in accord with the structure.[8] The last three ions were all the largest in their groups, with 165 dominating. They are in accord with the two different end-groups occurring in this compound. However, the ion at 181 m.u. also expected was insignificant.

The monoepoxide of cryptoxanthin gave a MS: 568 (20%, M^+); 550 (5% [M-18]); 488 (15% [M-80]); 476 (7% [M-92]); 338 (15%); 313 (20%); 273 (70%); 256 (100%); 221 (35%); 205 (70%); 181 (55%); 165 (90%). The molecular ion and the losses of 18 and 80 m.u. support the structural assignment. The peaks at 205 and 165 were the largest in their respective groups, dominating those at 221 and 181 m.u., which were not outstanding. These relationships may indicate a 5′,6′-epoxide for this pigment.[8]

Cryptoxanthin displayed a MS: 552 (100%, M^+); 534 (22% [M-18]); 460 (45% [M-92]); 446 (54% [M-106]). The principal ions in the upper region of the spectrum agree with those reported by Loeber.[12]

Cryptoflavin gave a MS: 568 (50%, M^+); 566 (7% [M-2]); 550 (4% [M-18]); 488 (100% [M-80]); 476 (20% [M-92]); 475 (20%); 422 (20%); 349 (30%); 336 (80%); 221 (150%); 205 (375%); 165 (375%). The molecular ion and the major fragments are as expected,[8]

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[10] BUDZIKIEWICZ, H., BREZINKA, H. and JOHANNES, B. (1970) *Monatsh. Chem.* **101**, 579.

[11] SCHWIETER, U., BOLLIGER, R., CHOPARD-DIT-JEAN, L. H., ENGLERT, G., KOFLER, M., KÖNIG, A., PLANTA, C. V., RÜEGG, R., VETTER, W. and ISLER, O. (1965) *Chimia* **19**, 294.

[12] LOEBER, D. E., RUSSEL, S. W., TOUBE, T. P., WEEDON, B. C. L. and DIMENT, J. (1969) *J. Chem. Soc.* **C**, 429.

the strong M-80 ion in particular indicating a 5,6 or 5,8 oxide. Ion *m/e* 165 was outstanding within its group and 205 was a strong isolated peak, while *m/e* 221 was relatively insignificant and 181 absent. These relationships may indicate a 5′,8′-epoxide and are in accord with similar conclusions drawn for the monoepoxide of cryptoxanthin above.

The less polar diol fraction contained lutein, *cis*-lutein, isolutein and violaxanthin. When cochromatographed with standard pigments isolated from spinach and avocado, they each gave a single spot. The isolated lutein gave a MS: 568 (20%, M^+); 566 (5% [M-2]); 550 (100% [M-18]); 536 (15%); 534 (10%); 532 (10% [M-2 × 18]); 476 (5% [M-92]); 462 (2% [M-106]); 458 (18% [M-92-28]); 444 (15% [M-106-18]). The spectrum contains the molecular and main fragment ions expected,[8] resembling closely the spectra reproduced by Budzikiewicz[10] and Enzell.[13]

A complex chromatogram was obtained after acetylation of the lutein for 3 hr, indicating the presence of the intermediary monoacetates. After acetylation for 24 hr, only the diacetate was obtained. The most polar fraction contained the furanoxide of lutein, chrysanthemaxanthin, which did not separate by cochromatography from the pigment prepared through the acidulation of isolutein. Neoxanthin appeared in two forms, the less polar being identical with spinach neoxanthin.

The pigment gave a MS with 600 (35%, M^+); 582 (31% [M-18]); 564 (100% [M-2 × 18]); 520 (24% [M-80]); 490 (48% [M-18-92]); 484 (60% [M-80-2 × 18]); 221 (360%); 165 (2400%). The molecular ion and fragment ions are in accord with the structure and with the spectrum reported by Budzikiewicz.[10] Ions at 205 and 181 m.u. were insignificant and 165 was 3 times more intense than 221, in contrast to expectation (221, 181 intense). Thus, caution is indicated in the interpretation of these ions.

Neoxanthin was preceded by neochrome, which could be identified through cochromatography with the pigment prepared by acidulation of neoxanthin. The peel carotenoids were qualitatively the same as the pulp carotenoids, with the exception of the carbonyl carotenoid. This carotenoid had a broad maximum at 454 nm and showed a shift of only 9 nm after reduction. Its behaviour was identical with that of synthetic echinenone (Hoffmann La Roche) from which it did not separate upon TLC, and which has exactly the same reduction spectrum (420, 445, 474 nm) in ethanol.

The concentration of the carotenoids was 20–25 μg/g fresh matter in the pulp and about 100–120 μg/g fresh matter in the peel. The quantitative composition of pulp and peel is given in Table 2. In the pulp, the hydrocarbon fraction represents about 54% compared with 28% monols and 18% diols and polyols. The predominant pigment in the hydrocarbon fraction is all-*trans*-β-carotene representing about 60% of the fraction, γ-carotene, mutatochrome and the carbonyl representing about 10%. Phytoene, as well as ζ-carotene are found in traces and phytofluene as a minor pigment. In the monol fraction, cryptoxanthin predominates, with the epoxides representing 4–8% of the fraction. In the last fraction, the diols and polyols, the major pigments are lutein, violaxanthin and neoxanthin.

In peel, the hydrocarbon fraction represents about 65% compared with 7% monols and 28% diols plus polyols. The predominant pigment in the hydrocarbon fraction is β-carotene, representing about 70% of this fraction. Phytoene and phytofluene are present in very small amounts. The other hydrocarbons represent about 7% of the fraction. In the monol fraction, the ratio between cryptoxanthin and its epoxide is 10:1, as in the pulp. In the diol-polyol fraction, the major pigments are lutein, violaxanthin and neoxanthin, lutein consti-

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[13] Enzell, C. R., Francis, G. W. and Liaaen-Jensen, S. (1969) *Acta Chem. Scand.* **23**, 727.

tuting about half of this fraction. The amount of violaxanthin present was about half that of the lutein.

The major discernible pigment pattern of the loquat fruit is β-carotene (33·0 %), γ-carotene (6·1 %), cryptoxanthin (22·0 %), lutein (3·2 %), violaxanthin (2·9) and neoxanthin (4·9 %). It is of interest taxonomically to compare this fruit with other genera of the *Rosaceae* in which intergeneric differences have been observed. It is known that plants of the genus *Rosa* synthesize rubixanthin whereas other genera of the *Rosaceae* do not.[14,15] Loquat (genus *Eriobotrya*) was found to have a carotenoid pattern similar to that of apricot (*Prunus armeniaca*),[16,17] except for the absence of lycopene in *Eriobotrya*.

TABLE 2. QUANTITATIVE CAROTENOID DISTRIBUTION IN THE PULP AND IN PEEL OF THE LOQUAT FRUIT *Eriobotrya japonica* GOLDEN NUGGET VARIETY

	Pulp % of total carotenoids*	Peel % of total carotenoids		Pulp % of total carotenoids*	Peel % of total carotenoids
Unknown (a + b)	0·5	Traces	Cryptoxanthin epoxyfuranoxide	0·9	0·5
Phytofluene	0·5	0·3	Cryptoflavin	1·6	0·6
cis-neo-β-Carotene	5·0	5·3	Lutein	3·2	13·0
β-Carotene	33·0	50·0	*cis*-Lutein	0·7	1·3
γ-Carotene	6·1	5·2	Isolutein	1·7	2·2
Mutatochrome	1·4	1·4	Violaxanthin	2·9	4·3
Carbonyl	5·5	3·0	Crysanthemaxanthin	0·9	0·6
γ-Carotene-like	1·6	0·2	Luteoxanthin	1·4	2·5
Cryptoxanthin diepoxide	2·2	0·4	Neochrome	1·0	0·5
Cryptoxanthin monoepoxide	2·8	0·6	Neoxanthin	4·9	3·2
Cryptoxanthin	22·0	4·7	Neoxanthin-like	0·2	0·4

* Expressed as β-carotene.

Isomeric forms of the same pigment were determined together when they appeared in small quantities. Minor pigments were not recorded.

It is of interest that the carotenoid pattern is more similar between these two genera of different subfamilies—*Eriobotrya* from the subfamily *Maloideae* and *Prunus armeniaca* from the subfamily *Prunoideae*—than it is among the other genera of *Maloideae*[18] or even among the *Prunoideae* species.[16,19,20]

It should be noted that echinenone has previously been identified only in algae.[21] It is unusual for cryptoxanthin and most of the possible epoxide and furanoxide derivatives to

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[14] GOODWIN, T. W. (1956) *Biochem. J.* **62**, 346.

[15] GOODWIN, T. W. (1970) in *The Biochemistry of Fruits and their Products* (HULME, A. C., ed.), Vol. I, p. 305, Academic Press, New York.

[16] KATAYAMA, T., NAKAYAMA, T. O. M., LEE, T. H. and CHICHESTER, C. O. (1971) *J. Food Sci.* **36**, 804.

[17] CURL, A. L. (1960) *Food Res.* **25**, 190.

[18] VALADON, L. R. G. and MUMMERY, R. S. (1967) *Ann. Bot.* **31**, 497.

[19] CURL, A. L. (1959) *Food Res.* **24**, 413.

[20] CURL, A. L. (1963) *J. Food Sci.* **28**, 623.

[21] KRINSKY, N. I. and GOLDSMITH, T. H. (1960) *Arch. Biochem. Biophys.* **91**, 271.

occur together; however, this group has been identified in citrus.[3,22–24] In algae (*Xanthophiceae*), only the 5′,6′-monoepoxide and the diepoxide of cryptoxanthin were found.[25] According to the MS, the main monoepoxide was 5′,6′- while the other was 5,6- or possibly 5′,6′-. The latter was not further analysed.

The group of lutein, *cis*-lutein, isolutein is very widespread and is detectable in fruits, flowers and leaves. The triol group was not as complex as that described by Curl in apricot, who found a more complex distribution and pigments such as trolliflor, persicaxanthin. A neoxanthin-like pigment was present in trace amounts and could not be analysed.

The differences between pulp and peels consisted mainly in the higher concentration of the pigments—about 5 times more in the peel. The unique qualitative difference was the carbonyl pigment, which in the peel resembled echinenone. The differences between the proportion of the main pigments suggest that in the peel the biosynthetic pathway is more developed.

The ratio of carotene-cryptoxanthin-lutein-neoxanthin 33:22:3:5 in the pulp compared with a ratio of 57:5:13:4 in the peel is interesting. The ratio between cryptoxanthin and lutein could be interpreted as though cryptoxanthin were the precursor of lutein. This is in accord with the work of Katayama *et al.*[16] Using labeled [2^{14}C]MVA, they found in apricots that β-carotene, cryptoxanthin and lutein were strongly labeled and suggested that lutein is formed from β-carotene via cryptoxanthin.

EXPERIMENTAL

Materials. The fruits were obtained from the Experimental Station of the Volcani Institute, Beit Dagan. They were fully mature in April–May. Pulp and peels were analysed separately.

Pigment extraction and saponification. The pigments were extracted and saponified as previously described.[2] Batches of about 1 kg. endocarp were homogenized in an Ultra Turrax homogenizer (Type T.P. 18/2N Janke & Kunkel) with acetone, in the presence of B.H.T. The extraction was repeated until the residue was colourless. The pigments were transferred to ether and saponified in the cold overnight. The alkali concentration of the mixture was 5%.

Chromatography. The chromatographic separation of the pigments was performed using a combination of column and TLC. The first separation of the extract was on a column of MgO-Hyflo Super Cel (1:1, w/w) (Seasorb 43-Magnesia FMC West Vaco and Hyflo Super Cel Johns Manville). The column was developed stepwise with increasing amounts of acetone in petrol. 60–80°. The hydrocarbons were eluted with 1–5% acetone in petrol. With 10% acetone, three groups separated that were later mechanically cut, the epoxides of cryptoxanthin, the cryptoxanthin group and the most polar diols plus polyols. The last group was rechromatographed on the same adsorbent and could be separated into two groups, the less and the more polar xanthophylls, by developing with 10% acetone in petrol.–EtOH (99:1) and 10% acetone in petrol.–EtOH (97:3). The hydrocarbon fraction was further rechromatographed on neutral alumina, activity I, which was developed with increasing concentrations of Et_2O in petrol. (up to 50%) and finally with EtOH. Each eluate was further purified as follows: The less polar fraction, eluted with 5% Et_2O, contained phytoene, a yellow unknown pigment and phytofluene, and was rechromatographed on Alumina Brockmann grade III. Aliquots of 2 ml were collected and examined spectrophotometrically. The other fractions were purified through multiple successive rechromatography on thin layer plates using adsorbents MgO–kieselgur, alumina and silica gel G with the solvent systems specified in Table 1. Cochromatography with synthetic natural pigments were performed on the most suitable absorbent. Reference pigments were prepared from citrus, tomato, spinach, avocado and carrots. The synthetic pigments were gifts from Hoffman La Roche.

Chemical tests. The chemical tests used were described previously.[2] *The carbonyl test*, based on reduction with sodium borohydride, was monitored spectrophotometrically in order to observe the appearance of fine structure and the hypsochromic shift. The reduced pigment was also analysed chromatographically. *The epoxide test.* The epoxides were determined by treating an alcoholic solution of the pigment with 0·1 N HCl and observing the hypsochromic shift spectrophotometrically. In addition, thin layer chromatograms were exposed to HCl gas for a few minutes and the colour changes noted. An additional test was the *acetyla-*

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[22] Curl, A. L. (1962) *J. Food Sci.* **27,** 171.

[23] Curl, A. L. and Bailey, G. F. (1961) *J. Food Sci.* **26,** 442.

[24] Yokoyama, H. and Vandercook, C. E. (1967) *J. Food Sci.* **32,** 42.

[25] Stranky, H. and Harper, A. (1970) *Arch. Mikrobiol.* **71,** 164.

tion test,[26] which was performed as follows: To 100–200 μg of carotenoid in 1 ml of pyridine, 0·2 ml Ac_2O was added. The mixture was kept at room temp. in the dark and the course of acetylation was followed chromatographically at different time intervals. After 2–3 hr, it was possible to detect one monoacetate when monols were tested and a mixture of several acetates when the unsymmetrical diols were tested. In both cases, a single spot only was detected after 24 hr.

MS. MS were recorded on a Hitachi–Perkin–Elmer RMU-6E instrument. The samples (*ca.* 50 μg compound) were evaporated in a stream of N_2 and transferred as very concentrated solutions to the cavity of the microsample tubes when final evaporation in N_2 left the solid. This was rapidly inserted into the direct inlet system. Spectra were observed (sensitivity ×100), at 70 eV, samples being heated to 280–300°, with chamber temp. 250–280°. The heavy ions usually appeared only after several (3–10) min. Their masses were established by the combined use of the instrument's mass marker and, in most cases, heptacosafluorotributylamine as internal standard. Where peaks coincided, the contribution of heptacosa was calculated from a standard spectrum and deducted. As relative abundances varied strongly with time, they are quoted as percentage of the strongest peak in the high mass region to which principal attentions was given.

Absorption spectra. The absorption spectrum of each carotenoid was recorded in a Beckmann DB spectrophotometer from 220 to 550 nm. Quantitative determination was carried out using the method described by Davies.[27]

[26] KLEINIG, H. and REICHENBACH, H. (1970) *Arch. Mikrobial.* **74,** 223.

[27] DAVIES, B. H. (1965) in *Chemistry and Biochemistry of Plant Pigments* (GOODWIN, T. W., ed.), p. 489, Academic Press, New York.