Carotenoids and Related Compounds. Part 41.[1] Structure of Mytiloxanthin and Synthesis of a *cis*-Isomer

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Mytiloxanthin, an acidic pigment from the edible mussel, *Mytilus edulis*, yielded a diacetate on acetylation. Spectroscopic and other studies on the natural pigment and its derivative showed the former to be 3,3',8'-trihydroxy-7,8-didehydro-β,κ-caroten-6'-one (**1**). The absolute stereochemistry 3*R*, 3'*S*, 5'*R* is proposed.

Condensation of the trienedial (**23**) with the Wittig salt (**22**) gave the aldehydo ester (**24**) which was converted into the ethylenedioxy derivative (**25**). Condensation of (**25**) with the methyl ketone (**16**), and removal of the protecting group, gave the β-diketone (**27**) which reacted with the Wittig salt (**29**) to yield a desoxy-mytiloxanthin (**30**). A similar series of reactions based on the methyl ketone (**20**) led to a *cis*-isomer of mytiloxanthin.

In 1940 Sheer reported the isolation of two pigments from the mussel, *Mytilus californianus*.[2] One was believed to be zeaxanthin; the other was a new acidic carotenoid, mytiloxanthin. Subsequently two pigments in *M. edulis* were shown to be chromatographically identical with those from *M. californianus*. However, the 'zeaxanthin' was found to be the diacetylenic analogue, alloxanthin (**3**),[3] which has very similar chromatographic and visible light absorption properties.[4] In this paper we give details of our studies which led to the formulation of mytiloxanthin as (**1**).[5,6,‡]

Preliminary tests indicated that the production of red pigments in *M. edulis* was at a maximum during the summer months. Chromatography of the red fraction isolated from mussels collected at Southend during July and August gave two carotenoids, both of which were shown by precision mass spectrometry to have the molecular formula $C_{40}H_{54}O_4$. One had properties in good agreement with those reported by Sheer for mytiloxanthin; the isomer 'isomytiloxanthin', is the subject of the next paper.[7]

As noted by Sheer, the light absorption maximum of mytiloxanthin in ethanol underwent a hypsochromic shift on the addition of alkali, the original absorption being restored on acidification. The i.r. absorption spectrum contained bands due to hydroxy groups, and another ($\nu_{max.}$ 1 605 cm^{-1}) attributable to a hydrogen-bonded carbonyl group. It also exhibited the weak absorption band ($\nu_{max.}$ 2 170 cm^{-1}) associated with a polyene conjugated acetylenic linkage.[3]

The n.m.r. spectrum of mytiloxanthin included six bands resulting from methyl groups on sp^3 hybridised carbon atoms. Three of these (δ 1.14, 1.19, and 1.90) are characteristic of the acetylenic end group in alloxanthin (**3**).[4] The others (δ 0.85, 1.19, and 1.34) are similar to those (δ 0.84, 1.20, and 1.37) associated with the end group in capsorubin (**4**).[8] The n.m.r. spectrum also included an in-chain methyl band (δ 1.97), and two singlets which had not been observed previously with carotenoids: one

at δ 16.25 which was assigned to the proton in an enolic hydroxy group; the other at δ 5.82 which was assigned to the proton in the α-position of an enolised β-diketone (COC*H*=COH).

Acetylation of mytiloxanthin led to a di-acetate, $C_{44}H_{58}O_6$, together with small amounts of an anhydro mono-acetate, which, unlike mytiloxanthin and its diacetate, was readily eluted from alumina and is therefore not acidic. Reduction of mytiloxanthin, and of its diacetate, with an excess of sodium borohydride, gave a product with visible light absorption properties in good agreement with those to be expected[3] for a 7,8-acetylenic analogue of apo-8'-β-carotenol (**9**).[9]

The findings outlined above led to the provisional formulation of mytiloxanthin as (**1**) and of the diacetate as (**2**). The non-acidic anhydro monoacetate was assigned the related structure with the keto end group (**15**), formed by an intramolecular attack at C-3' by an enolate anion at C-8'.

Support for these conclusions was provided by the mass spectrum of mytiloxanthin. The fragmentation pattern included strong lines due to ions with the composition $C_8H_{15}O$ and C_8H_{13}; that the latter ion was formed from the former by elimination of the elements of water, was confirmed by observation of the appropriate metastable ion. These ions are attributed to transformations analagous to those observed with capsorubin (**4**) (see Scheme).[10]

The fragmentation pattern also indicated the formation of ions with the composition $C_{29}H_{37}O$ and $C_{11}H_{17}O$, due to the two alternative fissions of the 8',9'-bond in the di-keto tautomer of mytiloxanthin.

The above analysis assumes that the magnetic shielding of the ring methyl groups in a polyene ketone, such as capsorubin (**4**), will be similar in the enolic form of the corresponding β-diketone. To check this assumption Claisen-type condensations[1] were carried out between crocetin dimethyl ester[11] (**5**) and the methyl ketone (**16**),[12] and between methyl 8'-apo-β-carotenate (**10**)[13] and the methyl ketones (**16**)[1,12] and (**17**),[14] and with the trimethylsilyloxy derivative of (**18**).[14] These reactions led to the polyenes (**8**), (**12**), (**13**), and (**14**) respectively, all of which, like mytiloxanthin, exhibited n.m.r. bands near δ 5.8 and 16.3 due to two protons in the enolic β-diketone grouping (Table). The bands due to the methyl groups on the five-membered rings were similar to those due to the same groups in the related polyene ketones (**6**),[12] (**7**),[14] and (**12**).[12] Convincing evidence that mytiloxanthin contains an end group of the type **h**, with the two oxygen substituents on the five-membered ring *trans* to one another, as in capsorubin, was

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‡ See abstract and experimental section for naming of mytiloxanthin, and related keto-carotenoids, according to the recommendations of the IUPAC-IUB Commission of Biochemical Nomenclature (*Pure Appl. Chem.,* 1975, **41**, 407). The carbon atoms in carotenoids are numbered according to the same recommendations, as illustrated in (**1**) and (**9**). Other compounds are named and numbered according to standard conventions.
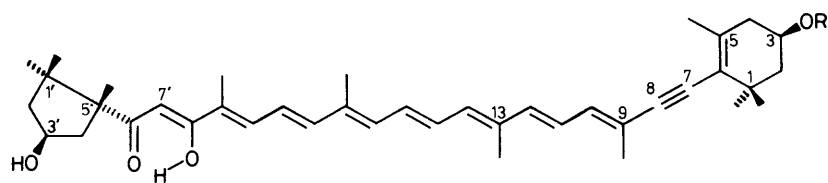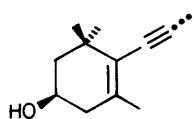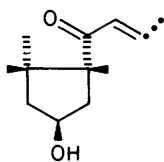
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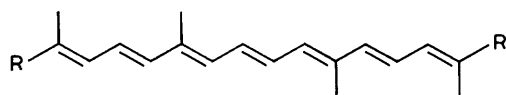
(3) R = a   (5) R = CO₂Me   (7) R = d
(4) R = b   (6) R = c   (8) R = e
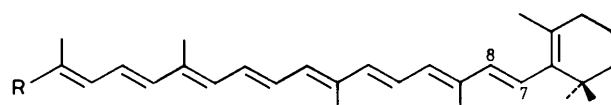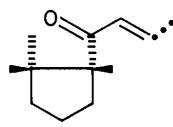

(9) R = CH₂OH   (11) R = c   (13) R = f
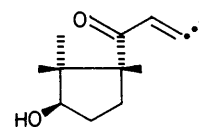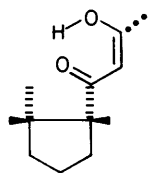(10) R = CO₂Me   (12) R = e   (14) R = g

a   b   c   d
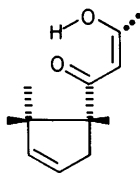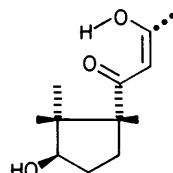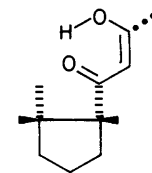
e   f   g   h
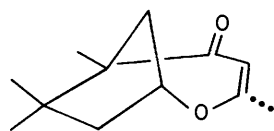
(15)
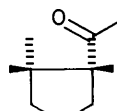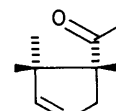
(16)   (17)
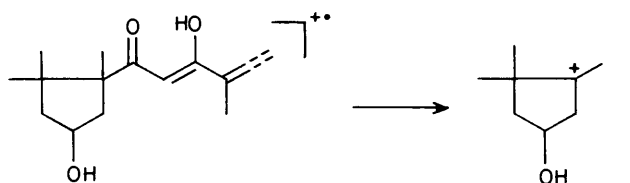
m/z 598·401   m/z 127·112

(18) R = H   (20) R = H
(19) R = SiMe₃   (21) R = SiMe₃
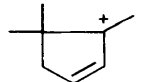
m/z 109·101

Scheme.
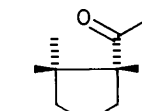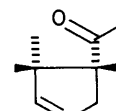
obtained subsequently by the unambiguous synthesis of tri-kentriorhodin.[1] This had an n.m.r. spectrum in which the bands associated with the end group **h** were identical with the corresponding bands in the spectrum of mytiloxanthin (Table).

The synthesis of mytiloxanthin was next examined. A partial condensation with the dial (23)[15] with the Wittig salt (22)[16]

(22)

(23)

(24) MeO₂C

(25) MeO₂C

(26) R = e

(27) R = e
(28) R = h

(29)

(30) R = e
(31) R = h

(32)
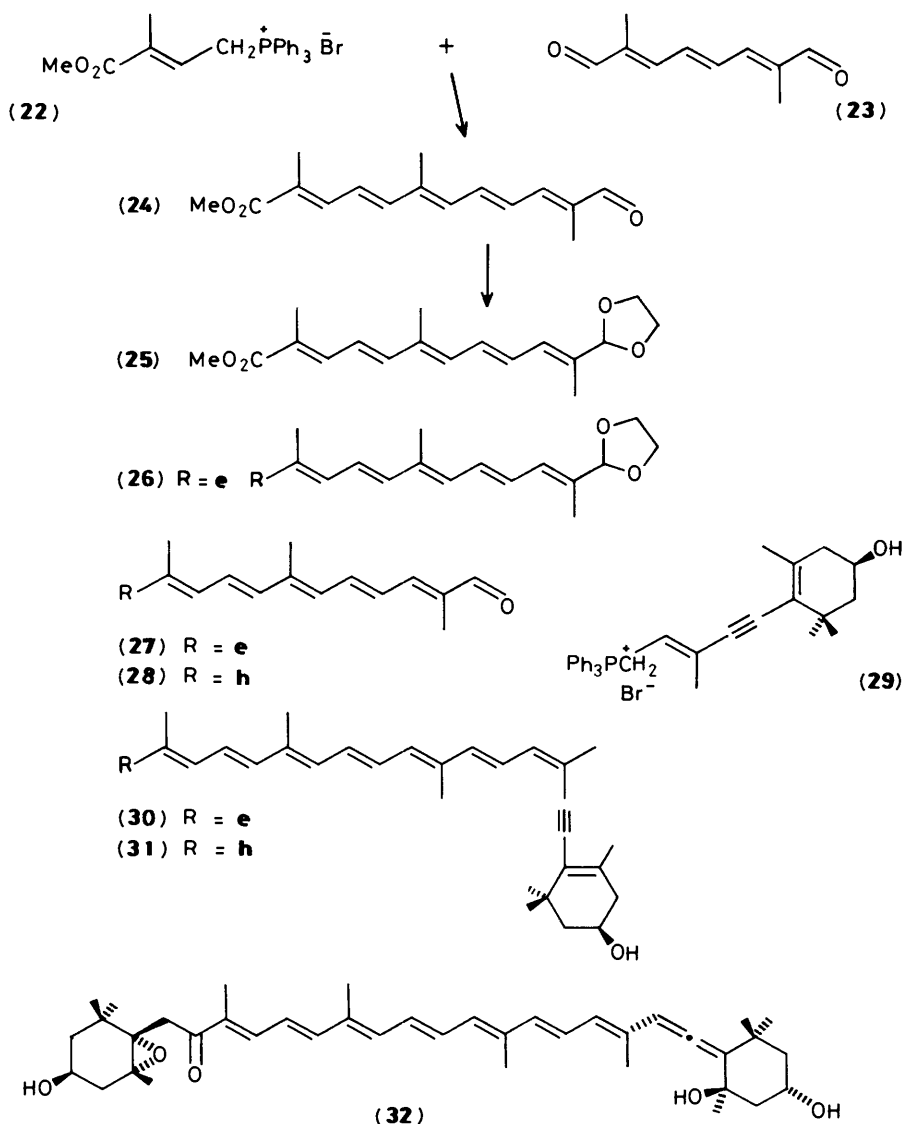
gave the required aldehydo ester (24), which was treated with ethanediol to yield the ethylenedioxy derivative (25). A Claisen-type condensation with the methyl ketone (16), and hydrolysis of the aldehyde protecting group, then furnished the β-diketone (27). This, on condensation with the Wittig salt (29), prepared by modification of the route developed earlier for the racemate,[4] gave the required 'desoxy-mytiloxanthin'. However, the position of its visible light absorption maximum indicated that condensation had been accompanied by extensive stereomutation, presumably to the cis-9 isomer (30) since this would be expected to be the most stable isomer thermodynamically.[4] Similar stereomutations have been observed during related Wittig condensations.[4]

Repetition of this sequence of reactions with (20),[14] after protecting the hydroxy group by trimethylsilylation, led to an isomer of mytiloxanthin which exhibited a cis-peak in the u.v. spectrum, and a visible light absorption maximum at a wavelength 7 nm shorter than that of the neutral pigment, as would be expected for the cis-9 isomer (31). Its electronic light absorption spectrum, and chromatographic properties, were identicial with those of a sample of natural mytiloxanthin which had undergone stereomutation on long storage.

It is conceivable that mytiloxanthin is a metabolite of fucoxanthin (32), present in the diet of the mussel. The possible

formation of the 3R-alloxanthin type end group from the allenic end group of fucoxanthin has been discussed previously.[17] A pinacollic rearrangement of the epoxide end group, analogous to that proposed for the biogenesis of capsorubin (4) and related pigments,[18] would give the β-diketone end group of mytiloxanthin. It is, therefore, suggested that (1) also represents the absolute configuration of mytiloxanthin. This is the same as the absolute configuration of the synthetic cis-isomer.

Experimental

The general comments in the Experimental section of the previous paper apply.

Isolation of Mytiloxanthin (1) *and Isomytiloxanthin.*—The body tissues from 3 000 specimens of *Mytilus edulis* (collected at Southend during July and August, 1971) were macerated in a blender and then extracted with acetone (5 l) for 48 h. The mixture was filtered and the solid was twice re-extracted with acetone. The acetone extracts were combined and evaporated under reduced pressure to remove the acetone. The aqueous residue was extracted repeatedly with ether, until the extracts were colourless. The ethereal extracts were combined, concentrated, dried, and evaporated. Column chromatography of

Table. Principal ^1H n.m.r. bands (in C^2HCl_3)

Compound	1-Me$_2$	5-Me	9-Me	13-Me	13'-Me	9'-Me	5'-Me	1'-Me	7'-H and/or 7-H	Enolic OH^b	Other bands
Alloxanthin (3)[4]	1.14, 1.19	1.90	1.99	1.94	1.94	1.99	1.99	1.19, 1.14			1.50 (s,^{b} 2 H)
Mytiloxanthin (1)	1.14, 1.19	1.90	1.97	1.97	1.97	1.97	1.20	1.34, 0.85	5.82	16.25	1.53 (m,^{b} 2 H)
Mytiloxanthin diacetate (2)	1.17, 1.20	1.91	1.98	1.98	1.98	1.98	1.25	1.30, 0.87	5.83	16.24	2.02 (s, 3 H, OAc) 2.04 (s, 3 H, OAc)
Trikentriorhodin[1]	0.85, 1.34	1.20	1.97	1.97	1.97	2.09			5.85	16.28	2.20^c (s, 3 H) 2.30^c (s, 6 H)
Capsorubin (4)[8]	0.84, 1.37	1.21	1.95	1.98	1.98	1.95	1.21	1.37, 0.84			
Bis-β-diketone (8)	0.88, 1.28	1.10	2.00	2.00	2.00	2.00	1.10	1.28, 0.88	5.88	16.38	
Diketone (6)[12]	0.85, 1.19	1.11	1.97	1.97	1.97	1.97	1.11	1.19, 0.85			
β-Diketone (12)	1.04, 1.04	1.71	1.98	1.98	1.98	1.98	1.12	1.18, 0.88	5.87	16.42	
Ketone (11)[12]	1.03, 1.03	1.72	1.97	1.97	1.97	1.97	1.11	1.18, 0.85			
β-Diketone (13)	1.04, 1.04	1.72	1.96	1.96	1.96	1.96	1.24	1.21, 0.92	5.90	16.22	
Isocapsorubin (7)[14]	0.84, 1.31	1.06	1.99	1.97	1.97	1.99	1.06	1.31. 0.84			
β-Diketone (14)	1.04, 1.04	1.72	1.98	1.98	1.98	1.98	1.06	1.21, 0.85	5.86	16.34	
β-Diketone (26)	0.88, 1.18	1.12	1.98	1.98	1.71				5.88	16.38	
β-Diketone (27)	0.88, 1.18	1.11	2.00	2.06	1.88				5.90	16.34	9.47 (s, 1 H, CHO)

^{a} All bands quoted (δ values) were singlets, unless indicated to the contrary, and had the expected relative intensities. The assignment of bands differing by less than 7 p.p.m. is arbitrary, and may have to be reversed. ^{b} Band due to hydroxy proton (s); removed after shaking the solution of the sample with deuterium oxide. ^{c} These two bands are due to the three methyl groups in the C-1, C-2, and C-3 position of the χ—end group.

the residue on silica gel (acetone–benzene) gave three fractions. The least polar fractions contained β-carotene and β-sitosterol. The most strongly adsorbed fraction contained only minor quantities of carotenoids, and was not examined further. The fraction of intermediate polarity contained two main carotenoids which were separated by t.l.c. on eight magnesium carbonate plates (50 × 50 cm) using 15% acetone in light petroleum as eluant. The coloured bands were scraped from the plates, the corresponding fractions were combined, and the pigments extracted from the absorbent with methanol. Evaporation of the two sets of extracts gave two pigments. The less polar (isomytiloxanthin) is described in the following paper. The more polar had a visible light absorption curve identical with that reported by Sheer[2] for mytiloxanthin isolated from *M. californianus*.

Further chromatography of the polar pigment on a column of silica gel (acetone–benzene), and crystallisation from benzene–light petroleum, gave mytiloxanthin (6 mg), m.p. 147 °C (evac. cap.); $\lambda_{max.}(CS_2)$ 496 nm; $\lambda_{max.}$(ethanol) 470 nm; $\lambda_{max.}$(benzene) 480 nm (ε 85 7000); $\nu_{max.}(CHCl_3)$ 3 680, 3 610, 2 950, 2 920, 2 850, 2 170, 1 605, 1 560, 1 460, 1 380, 1 100, 1 050, 1 030, and 970 cm^{-1}; $\nu_{max.}$(KBr; selected bands only) 2 170 and 965 cm^{-1}; δ, see Table; m/z 598.401 ($M^{+\cdot}$; Calc. for $C_{40}H_{54}O_4$: m/z 598.402, 23), 583 ($M - 15$, 1), 580 ($M - 18$, 3), 565 ($M - 15 - 18$, 1), 562 ($M - 18 - 18$, 0.5), 506 ($M - 92$, 4), 471 (0.5), 443 (1.5), 401.283 ($M - 197$; Calc. for $C_{29}H_{37}O$: m/z 401.284, 3.5), 197.117 ($M - 401$; Calc. for $C_{11}H_{17}O_3$ 197.118, 17), 179 ($M - 197 - 18$, 23), 155 (8.5), 127.112 (Calc. for $C_8H_{15}O$: m/z 127.112, 17), 119 (22), 109.101 (127 − 18, Calc. for C_8H_{13} 109.101, 90; m^* 93.5, 109^2/127 = 93.5), 105 (19), 92 (60), 91 (100), and 83 (52); c.d. (dioxane) 298 nm (Δε + 0.85), 254 nm (Δε − 1.71) and 216 nm (Δε − 1.71). Mytiloxanthin could not be eluted from an alumina t.l.c. plate with ethyl acetate.

Addition of an excess of 20% ethanolic potassium hydroxide to a solution of mytiloxanthan in ethanol resulted in a hypsochromic shift in the light absorption maximum to 455 nm. Subsequent addition of an excess of acetic acid restored the original light absorption curve, $\lambda_{max.}$ 470 nm.

An excess of sodium borohydride was added to a solution of mytiloxanthin in ethanol. The reaction was monitored spectroscopically and when complete (20 min) the solution had $\lambda_{max.}$ 455 and 428 nm.

Acetylation of Mytiloxanthin.—Acetic anhydride (0.4 ml) was added to mytiloxanthin (2 mg) in pyridine (0.5 ml), and the solution was kept at 20 °C for 48 h. Ether was added, and the ethereal solution was washed thoroughly with water, dried, and evaporated. Chromatography of the residue on a column of silica gel (acetone–benzene) gave the following two products.

(i) The less polar, *mytiloxanthin diacetate* (2) (1.3 mg), m.p. 121 °C (evac. cap.); $\lambda_{max.}$(ethanol) 473 nm; $\lambda_{max.}$(benzene) 483 nm; $\nu_{max.}$(KBr) 3 460, 2 960, 2 920, 2 860, 2 170, 1 735, 1 605, 1 560, 1 520, 1 460, 1 375, 1 260, 1 240, 1 095, 1 025, 970, and 800 cm^{-1}; δ, see Table; m/z 682.422 ($M^{+\cdot}$; Calc. for $C_{44}H_{58}O_6$: m/z 682.423, 1.1), 667 ($M - 15$, 0.5), 664 ($M - 18$, 0.5), 622 ($M - 60$, 28; m^* 567.5, 622^2/682 = 567.2), 607 ($M - 60 - 15$, 1.6; m^* 592.5, 607^2/622 = 592.4), 604 ($M - 60 - 18$, 0.6; m^* 586.5, 604^2/622 = 586.5), 590 ($M - 92$, 0.5), 562 ($M - 60 - 60$, 5.5; m^* 508.0, 562^2/622 = 507.8), 547 ($M - 60 - 60 - 15$, 7.2; m^* 532.5, 547^2/562 = 532.4), 530 ($M - 60 - 92$, 4.8), 443.295 (Calc. for $C_{31}H_{39}O_2$: m/z 443.295, 1), 239.128 (Calc. for $C_{13}H_{19}O_4$: m/z 239.128, 2), 179.106 (239 − 60; Calc. for $C_{11}H_{15}O_2$: m/z 179.107, 100), 169.122 (Calc. for $C_{10}H_{17}O_2$: m/z 169.123, 7), 123 (36), 119 (12), 109.101 (169 − 60; Calc. for C_8H_{13}: m/z 109.102, 22), 107 (11), 105 (1), and 91 (18). The diacetate was strongly absorbed on alumina t.l.c. plates, and was not eluted with ethyl acetate. Addition of 20% ethanolic potassium hydroxide to a soluton of the diacetate in ethanol caused a shift in $\lambda_{max.}$ to 455 nm; subsequent addition of an excess of acetic acid restored the original $\lambda_{max.}$ 470 nm. Addition of an excess of sodium borohydride to a solution of the diacetate in ethanol resulted, after 30 min, in a solution with $\lambda_{max.}$ 455 and 418 nm.

(ii) The more polar anhydro acetate (0.5 mg); $\lambda_{max.}$(ethanol) 473 nm; $\lambda_{max.}$(benzene) 484 nm; m/z 622 ($M^{+\cdot}$, 2.5), 607 ($M - 15$, 0.5), 562 ($M - 60$, 2), 547 ($M - 60 - 15$, 1.8), 530 ($M - 92$, 1.5), 179 (33), 123 (13), 122 (20), 119 (7), 109 (35), 107 (8), 105 (13), 91 (22), and 43 (100). Unlike mytiloxanthin and its diacetate, the anhydro acetate was eluted from an alumina t.l.c. plate with a mixture of ethyl acetate and benzene (30:70). The anhydro acetate was slowly reduced in ethanol with sodium borohydride; after 90 min the solution had $\lambda_{max.}$ 455 and 428 nm.

(5R,5'R)-8,8'-*Dihydroxy-κ,κ-carotene*-6,6'-*dione*   (8).—
Condensation[1] of dimethyl 8,8'-diapocarotene-8,8'-dioate

(crocetin dimethyl ester)[15] with (1R)-1-acetyl-1,2,2-trimethyl-cyclopentane[1,12] gave (ca. 60%) the bis-β-diketone as a red solid; λ_{max}.(ethanol) 470 nm; λ_{max}.(ethanolic NaOH) 454 nm; ν_{max}.(KBr) 2 920s, 2 557m, 1 760w, 1 605—1 560br s, and 962s cm^{-1}; δ, see Table; m/z 600.417 (M$^{+\cdot}$; Calc. for C$_{40}$H$_{56}$O$_4$: m/z 600.418, 46), 508 (M − 92, 14), 220 (5), 206 (12), 181 (25), 149 (13), 111 (100), 92 (32), 91 (53), 69 (75), 57 (27), 55 (78), 43 (55), and 41 (70).

An excess of sodium borohydride was added to a sample of the product in ethanol. After 15 min the solution had λ_{max}. 375, 352, and 338 nm.

(5′R)-8′-Hydroxy-β,κ-caroten-6′-one (12).—Condensation[1] of methyl 8′-apo-β-caroten-8′-oate[13] with (1R)-1-acetyl-1,2,2-trimethylcyclopentane,[1,12] and purification of the product by t.l.c. [silica gel, light petroleum (b.p. 60—80 °C) containing 2% acetone], and crystallisation from light petroleum (b.p. 60—80 °C), gave (82%) the β-diketone, m.p. 133—135 °C; λ_{max}.(ethanol) 470 nm; λ_{max}.(ethanolic NaOH) 452 nm; ν_{max}.(KBr) 2 920s, 2 860s, 1 590s, 1 560s, 1 520s, and 962s cm^{-1}; δ; see Table; m/z 568.429 (M$^{+\cdot}$; Calc. for C$_{40}$H$_{56}$O$_2$: 568.428, 55), 490 (3), 476 (M − 92, 15), 462 (M − 106, 2), 181 (46), 119 (32), 111 (82), 109 (25), 106 (40), 95 (38), 92 (100), 91 (100), 69 (92), 57 (35), 55 (80), and 41 (70).

An excess of sodium borohydride was added to a sample of the product in ethanol. After 10 min the solution had λ_{max}. 453, 429, and 408 nm.

(5′R)-8′-Hydroxy-2′,3′-didehydro-β,κ-caroten-6′-one (13).—Condensation of methyl 8′-apo-β-caroten-8′-oate with (4R)-4-acetyl-3,3,4-trimethylcyclopentene,[14] and purification of the product as in the previous experiment, gave (90%) the β-diketone, m.p. 145—147 °C; λ_{max}.(ethanol) 472 nm; λ_{max}.(ethanolic NaOH) 458 nm; ν_{max}.(KBr) 2 918m, 1 588s, 1 553s, 1 520s, and 962s cm^{-1}; δ, see Table; m/z 566.412 (M$^{+\cdot}$; Calc. for C$_{40}$H$_{54}$O$_2$: m/z 566.412, 75), 477 (M − 92, 29), 460 (M − 106, 5), 457 (M − 109, 4), 179 (100), 158 (65), 155 (50), 143 (34), 141 (33), 95 (50), 93 (95), 92 (100), 91 (100), 81 (46), 79 (48), 77 (76), 69 (89), 65 (100), 63 (84), 55 (85), 51 (92), 43 (100), and 41 (100).

An excess of sodium borohydride was added to a sample of the product in ethanol. After 10 min the solution had λ_{max}. 452, 426, and 406 nm.

(2′R,5′R)-8′-Hydroxy-2′-trimethylsilyloxy-β,κ-caroten-6′-one.—Condensation[1] of (1R,3S)-1-acetyl-1,2,2-trimethyl-3-trimethylsilyloxycyclopentane (19)[1] with methyl 8′-apo-β-caroten-8′-oate, and purification of the product by t.l.c., gave (65%) the β-diketone; λ_{max}.(ethanol) 474 nm; λ_{max}.(ethanolic NaOH) 458 nm; δ, see Table; m/z 656.461 (M$^{+\cdot}$; Calc. for C$_{43}$H$_{64}$O$_3$Si; m/z 656.463, 80). 641 (M − 15, 3), 564 (M − 92, 24), 540 (M − 106, 5). 437 (61), 109 (50), 106 (40), 105 (40), 92 (100), and 91 (100).

An excess of sodium borohydride was added to a sample of the product in ethanol. After 15 min the solution had λ_{max}. 452, 428, and 406 nm.

(2′R,5′R)-2′,8′-Dihydroxy-β,κ-caroten-6′-one (14).—0.2M Hydrochloric acid (10 ml) was added to the preceding trimethyl-siloxy compound (15 mg) in methanol (100 ml). When the reaction was complete, as judged by t.l.c., water was added and the product was isolated with ether. Preparative t.l.c. [silica gel, 11% acetone in light petroleum, (b.p. 60—80 °C)] gave the β-diketone (10 mg), m.p. 162—164 °C; λ_{max}.(ethanol) 474 nm; λ_{max}.(ethanolic NaOH) 456 nm; ν_{max}.(KBr) 3 460m, 1 600br m, 1 560s, 1 545s and 965s cm^{-1}; δ, see Table; m/z 584.422 (M$^{+\cdot}$; Calc. for C$_{40}$H$_{56}$O$_3$: m/z 584.432, 66), 566 (M − 18, 3), 492 (M − 92, 20), 478 (M − 106, 5), 149 (53), 139 (42), 127 (42), 119 (3), 109 (100), 106 (28), 105 (40), 94 (64), 92 (100), 91 (100), 73 (95), 71 (50), and 69 (100).

An excess of sodium borohydride was added to a sample of the product in ethanol. After 15 min the solution had λ_{max}. 452, 428, and 406 nm.

Methyl 12,12-Ethylenedioxy-2,6,11-trimethyldodeca-2,4,6,8,10-pentaenoate (25).—A solution of the aldehydo ester (24)[1] (260 mg), ethanediol (1 ml), and toluene-p-sulphonic acid (2 crystals) in benzene (20 ml) was boiled for 6 h under a Soxhlet extractor containing calcium hydride to remove the water found in the reaction. The solution was cooled, and saturated aqueous sodium hydrogen carbonate (10 ml) was added, and the product was isolated with ether. Preparative t.l.c. [silica gel, 10% acetone in light petroleum (b.p. 60—80 °C)] gave the acetal ester (250 mg) as a yellow solid; λ_{max}.(ethanol) 375 and 361 nm; ν_{max}.(KBr) 2 950w, 2 880w, 1 702s, 1 610w, 1 230s, 1 100m, 980m, and 952m cm^{-1}; δ, see Table; m/z 304.167 (M$^{+\cdot}$; Calc. for C$_{18}$H$_{24}$O$_4$: m/z 304.167, 6), 289 (M − 15, 4), 273 (M − 31, 5), 260 (M − 44, 27), 252 (74), 218 (50), 189 (100), 175 (70), 126 (100), 93 (75), 91 (100), 73 (100), 53 (43), and 45 (100).

Dilute hydrochloric acid was added to a sample of the acetal ester in ethanol. After 10 min the solution had u.v. light absorption properties similar to those of the aldehydo ester.

(5R)-12′,12′-Ethylenedioxy-8-hydroxy-12′-apo-κ-caroten-6-one (26).—Condensation of the preceding acetal ester (300 mg) with (1R)-1-acetyl-1,2,2-trimethylcyclopentane (300 mg), and purification of the product by preparative t.l.c. [silica gel, 10% acetone in light petroleum (b.p. 60—80 °C)], gave the β-diketone (230 mg) as a yellow solid; λ_{max}.(ethanol) 403 nm; λ_{max}.(ethanolic NaOH) 390 nm; ν_{max}.(KBr) 2 960s, 2 880m, 1 725m, 1 675m, 1 605s, 1 570br s, and 970m cm^{-1}; δ, see Table; m/z 426.276 (M$^{+\cdot}$; Calc. for C$_{27}$H$_{38}$O$_4$: m/z 462.277, 100), 382 (M − 44, 58), 354 (51), 315 (M − 111, 5), 287 (M − 139, 4), 111 (100), 91 (57), 73 (100), 69 (95), 55 (100), 44 (100), and 43 (100).

An excess of sodium borohydride was added to a sample of the product in ethanol. After 10 min the solution had λ_{max}. 378, 348, and 338 nm.

(3S,5R)-12′,12′-Ethylenedioxy-8-hydroxy-3-trimethylsilyl-oxy-12′-apo-κ-caroten-6-one.—Condensation[1] of the acetal ester (25) (100 mg) with (1R,3S)-1-acetyl-1,5,5-trimethyl-3-trimethylsilyloxycyclopentane[1] (60 mg), and purification of the product by preparative t.l.c. [silica gel, 10% acetone in light petroleum (b.p. 60—80 °C)] gave the β-diketone (70 mg) as a yellow oil, λ_{max}.(ethanol) 404 nm; λ_{max}.(ethanolic NaOH) 382 nm.

An excess of sodium borohydride was added to a sample of the product in ethanol. After 10 min the solution had λ_{max}. 365, 347, and 330 nm.

(5R)-8-Hydroxy-12′-apo-κ-caroten-6-on-12-al (27).—A solution of the ethylenedioxy derivative (26) (20 mg), and 2M-hydrochloric acid (3 ml) in methanol (15 ml) was stirred for 3 h. Water (20 ml) was then added, and the product was isolated with ether. Preparative t.l.c. [silica gel, 10% acetone in light petroleum (b.p. 60—80 °C)] gave the β-diketone aldehyde (15 mg) as a yellow solid; λ_{max}.(ethanol) 436infl and 421 nm; λ_{max}.(ethanolic NaOH) 420 nm; ν_{max}.(KBr) 3 460w, 3 040w, 2 958s, 1 670s, 1 602s, 1 570s, and 965m cm^{-1}; δ, see Table; m/z 382.250 (M$^{+\cdot}$; Calc. for C$_{25}$H$_{34}$O$_3$: m/z 382.251, 70), 271 (M − 111, 5), 243 (M − 139, 5), and 111 (100).

An excess of sodium borohydride was added to a sample of the product in ethanol. After 10 min the solution had λ_{max}. 376, 347, and 336 nm.

(3S,5R)-3,8-Dihydroxy-6-oxo-12′-apo-κ-caroten-12-al (28).—A solution of the ethylenedioxy trimethylsilyloxy derivative (50 mg) and 2M hydrochloric acid (3 ml) in methanol (15 ml) was stirred for 6 h. Isolation of the product as in the preceding

experiment, and preparative t.l.c. [silica gel, 15% acetone in light petroleum, (b.p. 60—80 °C)] gave the hydroxy β-diketone aldehyde (20 mg) as a red oil, $\lambda_{max.}$(ethanol) 420 nm; $\lambda_{max.}$(ethanolic NaOH) 414 nm.

An excess of sodium borohydride was added to a sample of the product in ethanol. After 15 min the solution had $\lambda_{max.}$ 365, 347, and 330 nm.

(4R)-2-trans-5-(4'-Hydroxy-2',6',6'-trimethylcyclohex-1'-enyl)-3-methylpent-2-en-4-ynyltriphenylphosphonium Bromide (29).—A solution of (4R,6R)-4-hydroxy-2,2,6-trimethylcyclo-hexanone[19] (1.45 g), dihydropyran (0.9 g) and toluene-p-sulphonic acid (10 mg) in ether (10 ml) was kept for 12 h. Water was added and the product was isolated in the usual way to give the tetrahydropyranyl derivative (2.3 g); $\nu_{max.}$(liq. film) 2 940, 1 700, 1 460, 1 350, 1 130, and 1 030 cm^{-1}; δ 1.00 (s, 6 H), 1.3 (d, J 7 Hz, 3 H), 1.5—2.2 (m, 10 H), 3.1 (m, 1 H), 3.5 (m, 1 H), 3.9 (m, 2 H), and 4.54 (m, 1 H). trans-3-Methylpent-2-en-4-yn-1-ol (0.95 g) in dichloromethane (15 ml) was added slowly to ethyl-magnesium bromide (from 0.48 g of magnesium) in ether (50 ml), and the mixture was stirred for 2 h. The preceding pyranyl ether in dichloromethane (15 ml) was added slowly and the mixture was stirred for 18 h. Saturated aqueous ammonium chloride (20 ml) was added, and the product was isolated with ether. Preparative t.l.c. [light petroleum (b.p. 60—80 °C)] gave the required diol (450 mg) as an oil; $\lambda_{max.}$(ethanol) 230 nm; $\nu_{max.}$(liq. film) 3 450, 2 970, 2 260, and 987 cm^{-1}; δ 1.06 (s 6 H), 1.16 (d, J 7 Hz, 3H), 1.4—1.6 (m, 10 H), 1.79 (s, 3 H), 3.02 (s, 2 H; lost on deuteriation), 3.80 (m, 1 H), 4.14 (d, J 7 Hz), 4.64 (m, 1 H), and 5.95 (m, 1 H); m/z 336.230 (M^{+}; Calc. for $C_{20}H_{32}O_4$: m/z 336.230, 7), 321 (M − 15, 10), 328 (M − 18, 4), and 85 (100).

A mixture of the diol (400 mg) and triphenylphosphonium bromide (400 mg) in chloroform (15 ml) was stirred in the dark for 36 h. The solvent was evaporated under reduced pressure and the residue was triturated with ether to give the Wittig salt as a pale yellow solid which was used without further purific-ation. Its structure was confirmed by reaction with the triene dial (23) to give the (3R)-isomer of the known 9-trans-3-hydroxy-7,8-didehydro-12'-apo-β-caroten-12'-al.[4]

(3R,5'R)-3,8'-Dihydroxy-7,8-didehydro-β,κ-caroten-6'-one (Desoxy-mytiloxanthin) (30).—Potassium hydroxide (500 mg) was dissolved in water (1 ml) and propan-2-ol (10 ml). A portion (2 ml) of the solution, and (3R)-5-(4'-hydroxy-2',6',6'-trimethylcyclohex-1'-enyl)-3-methylpent-2-en-4-ynyltriphenyl-phosphonium bromide (29) (80 mg), was added simultaneously over 20 min to a stirred solution of the aldehyde (27) (10 mg) in propan-2-ol (7 ml) at −30 °C in the dark. More of the potassium hydroxide solution (6 ml) was added in 1-ml portions every 5 min. The mixture was stirred at −30 °C for 1 h and then at 20 °C for 2 h. Water (10 ml) was added and the product was isolated with ether. Chromatography in silica gel [15% acetone in light petroleum (b.p. 60—80 °C)] indicated the presence of two pigments with only a small difference in R_F. Isolation of the major, more polar, pigment gave the desoxy-mytiloxanthin (4 mg) as a red solid; $\lambda_{max.}$(ethanol) 466 nm; $\lambda_{max.}$(ethanolic NaOH) 451 nm; $\nu_{max.}$(KBr) 3 440, 2 920, 2 870, 2 170, 1 740, 1 680, and 963 cm^{-1}; m/z 582.407 (M^{+}; Calc. for $C_{40}H_{54}O_3$: m/z 582.407, 38), 564 (M − 18, 3), 516 (4), 490 (M − 92, 6), 415 (7), 181 (30), 174 (26), 154 (35), 111 (100), 109 (100), 95 (48), 94 (100), and 91 (100).

An excess of sodium borohydride was added to a sample of the product in ethanol. After 20 min the solution had $\lambda_{max.}$ 451 and 424 nm.

The visible light absorption data suggest that the condens-ation product consisted mainly of the 9-cis isomer.

9-cis-Mytiloxanthin (31).—Condensation of the aldehyde (28) (15 mg) with the Wittig salt (29) (100 mg), and isolation of the product as in the previous experiment, gave two products of similar R_F. Isolation of the major, more polar, product gave 9-cis-mytiloxanthin (2 mg); $\lambda_{max.}$(ethanol) 463 and 364 nm; $\lambda_{max.}$(ethanolic NaOH) 448 nm; m/z 598.400 (M^{+}; Calc. for $C_{40}H_{54}O_4$: m/z 598.402, 22), 580 (M − 18,4), 562 (M − 18 − 18, 1), 506 (M − 92, 4), 368 (7), 207 (11), 197 (6), 183 (6), 179 (13), 143 (18), 137 (47), 127 (7), 118 (65), 109 (42), 92 (100), and 91 (98).

The visible light absorption spectrum of a sample of natural mytiloxanthin after prolonged storage was superimposable on that of the synthetic product. There was no separation of the two samples on mixed t.l.c. on silica gel using either 20% acetone in light petroleum, or benzene–ethyl acetate–ethanol (10:2:1), as eluant.

An excess of sodium borohydride was added to a sample of the synthetic product in ethanol. After 15 min the solution had $\lambda_{max.}$ 450, 426, and 404 nm.

References
1 Part 40, preceding paper.
2 B. T. Sheer, J. Biol. Chem., 1940, 136, 275.
3 S. A. Campbell, A. K. Mallams, E. S. Waight, B. C. L. Weedon, M. Barbier, E. Lederer, and A. Salaque, Chem. Commun., 1967, 941.
4 A. J. Davies, A. Khare, A. K. Mallams, R. A. Massy-Westropp, G. P. Moss, and B. C. L. Weedon, J. Chem. Soc., Perkin Trans. 1, 1984, 2147.
5 A. Khare, G. P. Moss, and B. C. L. Weedon, Tetrahedron Lett., 1973, 3921.
6 A. K. Chopra, G. P. Moss, and B. C. L. Weedon, Chem. Commun., 1977, 467.
7 A. Khare, G. P. Moss, and B. C. L. Weedon, J. Chem. Soc., Perkin Trans. 1, following paper.
8 R. D. Bowden, R. D. G. Cooper, C. J. Harris, G. P. Moss, and B. C. L. Weedon, J. Chem. Soc., Perkin Trans. 1, 1983, 1465.
9 R. Rüegg, M. Montavon, G. Ryser, G. Saucy, U. Schwieter, and O. Isler, Helv. Chim. Acta, 1959, 42, 854.
10 J. Baldas, Q. N. Porter, A. P. Leftwick, R. Holzel, B. C. L. Weedon, and J. Szabolcs, Chem. Commun., 1969, 415.
11 O. Isler, H. Gutmann, M. Montavon, R. Rüegg, G. Ryser, and P. Zeller, Helv. Chim. Acta, 1957, 40, 1242.
12 M. S. Barber, L. M. Jackman, C. K. Warren, and B. C. L. Weedon, J. Chem. Soc., 1961, 4019.
13 O. Isler, W. Guex, R. Rüegg, G. Ryser, G. Saucy, U. Schwieter, M. Walter, and A. Winterstein, Helv. Chim. Acta, 1959, 42, 862.
14 A. Rüttimann, G. Englert, H. Mayer, G. P. Moss, and B. C. L. Weedon, Helv. Chim. Acta, 1983, 66, 1939.
15 H. Mayer and O. Isler, in 'Carotenoids,' O. Isler (ed.), Birkhäuser, Basel, 1971.
16 E. Buchta and F. Andree, Chem. Ber., 1960, 93, 1349.
17 B. C. L. Weedon, Rev. Pure and Appl. Chem., 1970, 20, 51.
18 M. S. Barber, L. M. Jackman, C. K. Warren, and B. C. L. Weedon, Proc. Chem. Soc., 1960, 19; J. Chem. Soc., 1961, 4019.
19 H. G. W. Leuenberger, W. Boguth, E. Widmer, and R. Zell, Helv. Chim. Acta, 1976, 59, 1832.