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Bacterial Carotenoids

XXXII.* C_{50}-Carotenoids 6.* Carotenoids from *Corynebacterium poinsettiae* Including Four New C_{50}-Diols

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Dedicated to Dr. O. Isler on the occasion of his 60th birthday

The carotenoids produced in altogether 700 l of cultures of *Corynebacterium poinsettiae* Starr and Pirone, grown with varying thiamine supply, have been investigated. Complete extraction of the carotenoids was effected by lysozyme treatment prior to solvent extraction. Four new C_{50}-diols were isolated: bisanhydrobacterioruberin (*4*), 3,4,3′,4′-tetrahydro-bisanhydrobacterioruberin (*9*), 1′-hydroxy-1′,2′-dihydro-2-isopentenyl-2′-(hydroxy-isopentenyl)-torulene (*12*) and 2-(dihydroxy-isopentenyl)-2′-isopentenyl-β-carotene (*15*) and a C_{45}-mono-ol, 2-isopentenyl-3,4-dehydro-rhodopin (*20*). The structures followed from spectroscopic evidence (visible light, IR, NMR, and mass spectra) obtained for the natural carotenols and various acetylated, silylated and dehydrated derivatives.

In addition lycopene (C_{40}) was isolated, and three minor carotenoids, referred to as c.p. 450-mono-ol, c.p. 473-mono-ol, and c.p. 435, were partly characterized.

The carotenoids with supernumerary carbon skeleton isolated in the present work conform in principle with the substitution pattern encountered in other C_{50}-carotenoids.

Corynebacterium poinsettiae Starr and Pirone is an aerobic, mobile, Gram-positive, rod-shaped, phytopathogenic bacterium of taxonomically uncertain position, which requires thiamine for reproduction.[1–4] Low thiamine concentrations, supporting only moderate growth, result in pink cultures, whereas at higher thiamine levels the pigmentation is yellow-orange.[4]

Spirilloxanthin (*1*)[5] and more tentatively lycoxanthin (*2*)[6] have been identified as the main carotenoids of red cells. Orange-yellow cells contained in addition a dominant carotenoid considered to be cryptoxanthin (*3*). From these results Starr and Saperstein[4] suggested that thiamine may be active in

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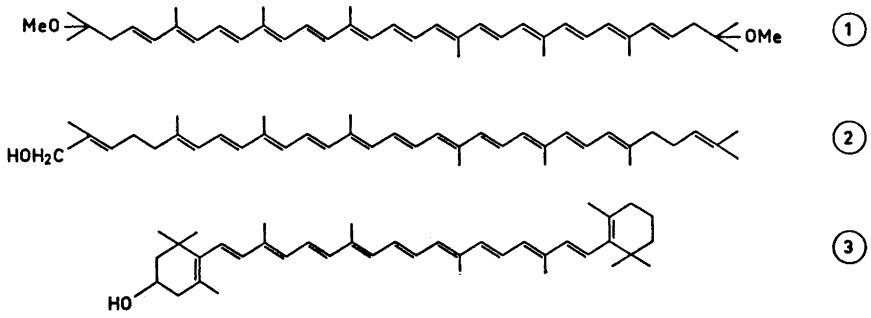
some metabolic process which results in cyclization of the carotenoids. At that time the oxygen substituents in spirilloxanthin (*1*) and lycoxanthin (*2*) were assumed to be in 3 (3′)-positions.



Corynebacterium poinsettiae was used by Kunisawa and Stanier [7] in studies performed to demonstrate the general, protective effect of carotenoids against photodynamic destruction. Carotenoid synthesis was suppressed in a white mutant obtained by ultraviolet irradiation or by growth in the presence of diphenylamine. In both cases the coloured carotenoids were largely replaced by phytoene and phytofluene.

In a reinvestigation to check the claimed occurrence of methoxylated carotenoids outside the photosynthetic bacteria the previous identifications of the coloured carotenoids were disproved.[8] Further structural studies were later undertaken,[9] but led at that time to no plausible structures.

Table 1. Carotenoid yield and composition of various cultures of *Corynebacterium poinsettiae*.

Carotenoid	In % of total carotenoid					
	Lot 1	Lot 2	Lot 3	Lot 4	Batch 1	Batches 2+3+4
Lycopene (*22*)						6.0
C.p. 435 (*27*)					0.8	0.6
C.p. 482 (*20*)						20.0
C.p. 450-mono-ol (*23*)						2.5
C.p. 473-mono-ol (*25*)						0.4
C.p. 470 (*9*)	2					3.5
C.p. 496 (*4*)	5	85			27.4	40.0
C.p. 473 (*12*)	46	15	50	30	16.8	17.0
C.p. 450 (*15*)	47		50	70	32.0	10.0
Undefined components					22.0	
Total in mg	0.34	0.04	0.02	0.04	9.2	108
Culture in l	1	0.2	0.2	0.2	170	510

Large scale cultivations and improved methods including NMR and mass spectrometry have now permitted structural assignments of the major carotenoids here isolated from *Corynebacterium poinsettiae*, four C_{50}-diols (*4*, *9*, *12* and *15*), a C_{45}-mono-ol (*20*)[10] and lycopene.

RESULTS AND DISCUSSION

The effect of thiamine on the carotenoid synthesis [4] was confirmed using small cultures; see Experimental part. For structural studies mass cultivation in four batches, each of 170 l, were carried out. One batch, grown with low thiamine supply, was analysed separately; the other three (two with low and one with high thiamine supply) jointly; see results in Table 1.

Complete pigment extraction, previously not achieved,[4,9] was effected by enzymatic treatment of the cells with lysozyme prior to solvent extraction.

C. p. 496 = bisanhydrobacterioruberin (*4*)

The pink pigment referred to as c.p. 496 and previously considered to be spirilloxanthin (*1*),[4] was assigned structure *4* (= bisanhydrobacterioruberin [11]). Crystalline c.p. 496, m.p. 170–171°C, yield *ca.* 6 mg, exhibited absorption

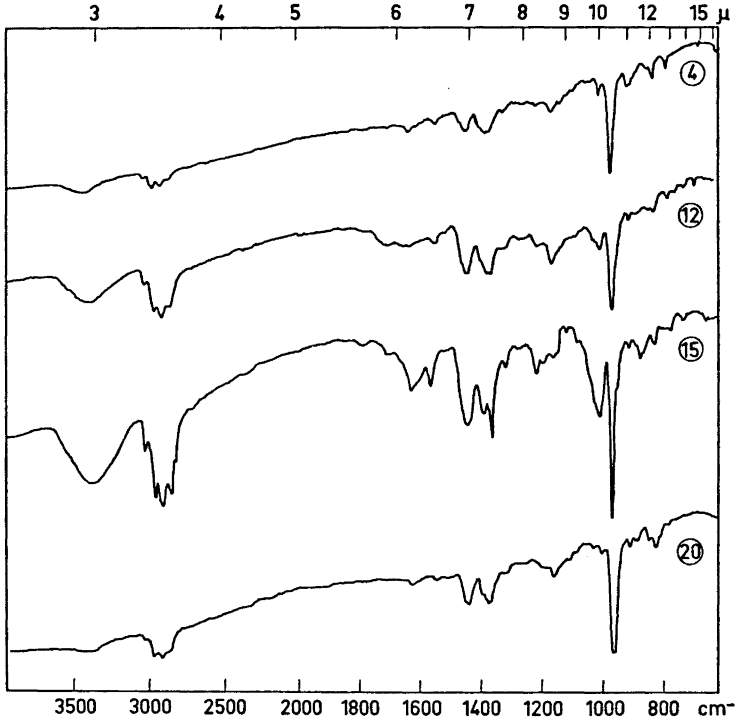
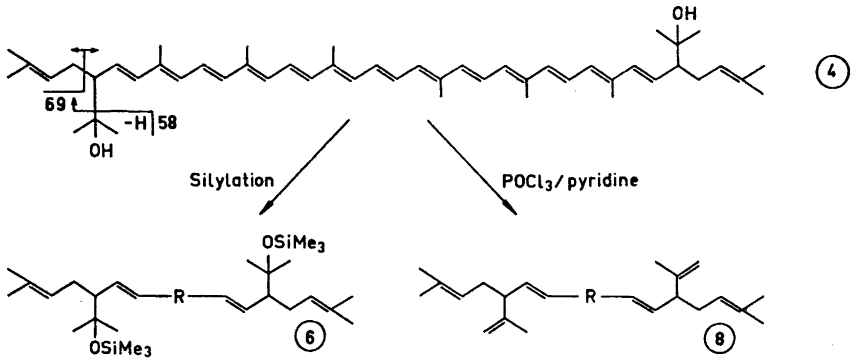


Fig. 1. Infrared spectra (KBr) of c.p. 496 (*4*), c.p. 473 (*12*), c.p. 450 (*15*), and c,p. 482 (*20*)

in visible light corresponding to an aliphatic tridecaene chromophore. Functional groups susceptible to hydride reduction were absent, as were primary, secondary, and allylic hydroxyl groups according to acetylation evidence and treatment with acidic chloroform.[12] The course of low-temperature silylation [11] to a mono (*5*) and di (*6*) trimethylsilyl ether revealed the presence of two tertiary hydroxy groups, confirmed by IR absorption at 1140 and 905 cm^{-1} (Fig. 1) and on dehydration with phosphorus oxychloride,[5] which provided a mono (*7*) and a bisanhydro (*8*) derivative, both with tridecaene chromophores. The bisanhydro derivative (*8*) exhibited characteristic IR absorption for terminal methylene at 885 cm^{-1}. The dehydration reaction has been discussed elsewhere.[11] Isomerization of the terminal double bonds of *8* into conjugation could not be effected with alkali.



The mass spectrum of c.p. 496 (*4*) had its molecular ion at *m/e* 704, confirmed by common losses of 92, 106, and 158 mass units from the polyene chain.[13,14] Elimination of higher analogues of the toluene, xylene, and dimethyldihydronaphthalene species was not observed, disfavouring extra in-chain substituents.[15] Losses of 69 and 58 (acetone) mass units from the molecular ion and fragment ions were in agreement with the cleavages indicated on structure *4*, *cf.* Ref. 11. Here and in the following the direction of the arrows is towards the charged fragment. Also the NMR spectrum was readily accomodated by structure *4* (four in-chain and two in-chain/end-of-chain methyl groups, two identical isopropylidene groups and two identical pairs of *gem.* dimethyl at tertiary hydroxyl and two allylic methylene groups). Slight magnetic nonequivalence of the 16, 16′ and 17, 17′ methyl groups (τ 8.83 and 8.81) is ascribed to the asymmetric 2,2′-carbon atoms.

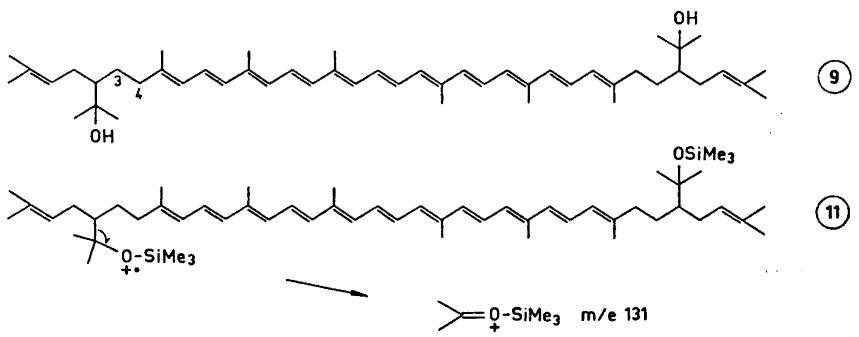
The extinction coefficient in visible light ($\varepsilon = 182\,000$ at 498 nm in acetone) was comparable with the highest one obtained for bacterioruberin ($\varepsilon = 194\,000$)[11] and surprisingly higher than for tridecaene chromophores in the C_{40}-series.[11] This irregularity has not yet been clearly demonstrated in the cyclic C_{50}-series, *cf. dehydrogenans*-P439 [16] and below.

Finally direct comparison of the stereoisomeric sets of c.p. 496, natural and semi-synthetic bisanhydrobacterioruberin of *Halobacterium salinarium* origin,[11] supported identity.

C.p. 470 = 3,4,3′,4′-tetrahydro-bisanhydrobacterioruberin (*9*)

C.p. 470 is identical with the carotenoid referred to as carotenoid A.[7] It is presumably identical with the carotenoid tentatively identified as lycoxanthin (*2*)[4] with which c.p. 470 agrees better in light absorption and adsorptive properties than the more abundant c.p. 473 described below.

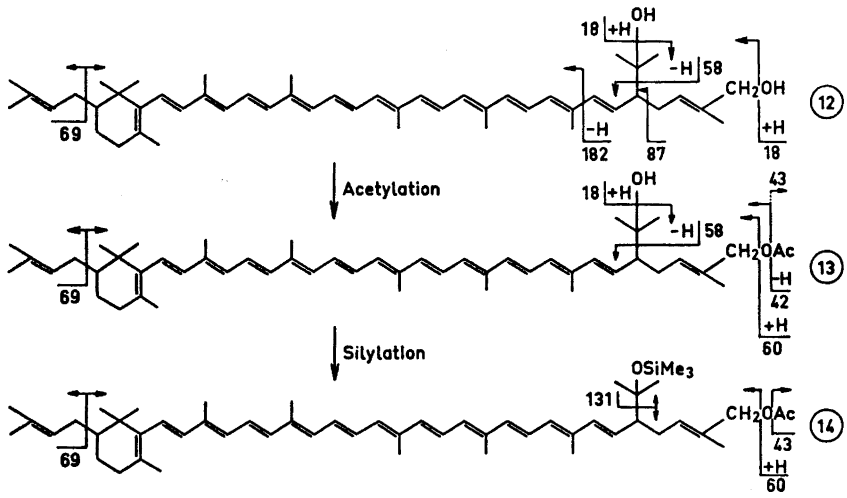
The visible absorption spectrum corresponded to an aliphatic undecaene chromophore. No acetates were formed on acetylation and a mono (*10*) and di (*11*) trimethylsilyl ether were obtained on silylation. The mass spectrum of c.p. 470 (*9*) exhibited characteristic peaks at *m/e* 708 (M, corresponding to $C_{50}H_{74}(OH)_2$), M−92, M−106 and *m/e* 69, but no peaks at M−58 and M−69. The di(trimethyl) silyl ether (*11*) had peaks at *m/e* 852 (M, corresponding to $C_{50}H_{74}(OSi(CH_3)_3)_2$), M−92, M−106 and *m/e* 131. This evidence, including lacking M−58 and M−69 peaks for *9*, *cf.* Ref. 14, is compatible with structure *9* (3,4,3′,4′-tetrahydro-bisanhydrobacterioruberin) for c.p. 470. 3,4,3′,4′-Tetrahydro-bisanhydrobacterioruberin (*9*) has been considered a plausible biosynthetic precursor of bisanhydrobacterioruberin (*4*).[11]



C.p. 473 (*12*)

C.p. 473 is identical with the carotenoid referred to as carotenoid B.[8] Repeatedly chromatographed and recrystallized c.p. 473, yield *ca.* 2 mg, m.p. 110°C, $\varepsilon = 103\,000$ at 477 nm in acetone, still contained non-carotenoid contaminants, although these were not evident in its spectra. Judged by the visible light absorption spectra the chromophore corresponded to the monocyclic dodecaene chromophore present in β-apo-2′-carotenol.[17] Functional groups susceptible to hydride reduction and hydroxyl groups in allylic position to the polyene chain were judged absent by the usual criteria. Acetylation gave a monoacetate (*13*) which gave a mono(trimethyl) silyl ether (*14*), demonstrating the presence of one primary or secondary and one tertiary hydroxy group in *12*. IR data (Fig. 1) confirmed the latter assignment (*tert.* hydroxyl at 1140 and 905 cm^{-1}) and indicated that the former hydroxy group was primary and allylic (1005 cm^{-1}, *cf.* lycoxanthin [6]), which was further confirmed by an NMR signal at τ 5.95 (2 H). The NMR spectrum further revealed four

in-chain (τ 8.03) and one in-chain/end-of-chain (τ 8.08) methyl groups and *ca.* two methyl groups attached to tertiary hydroxyl (τ 8.78). Signals at τ *ca.* 8.30 (three methyl groups) and 8.39 (one methyl group) could be attributed to one isopropylidene group (τ 8.32, 8.39), one end-of-chain methyl in a β-ring (τ 8.30) and one methyl group of the lycoxanthin-type end-group (τ 8.30). Singlets at τ 8.92 and 9.05 were ascribed to two magnetically non-equivalent *gem.* methyl groups of the substituted β-ring.



The mass spectra of the natural diol (*12*), the acetate (*13*), and the silyl ether (*14*) confirmed these assignments and permitted the allocation of the primary hydroxyl group to the aliphatic end-group. Thus the spectrum of c.p. 473 (*12*) had characteristic peaks at *m/e* 704 (M, corresponding to $C_{50}H_{70}(OH)_2$), M − 92, M − 106, and M − 158 and diagnostically useful peaks at M − 18, M − 58, M − 69, M − 87, and M − 182 corresponding to the cleavages indicated on structure *12*. The M − 182 ion had accurate mass corresponding to $C_{39}H_{54}$. The mass spectrum of the acetate (*13*) had characteristic peaks at *m/e* 746 (M, corresponding to $C_{50}H_{70}(OH)OCOCH_3$, M − 18, M − 42, M − 58, M − 60, M − 69, M − 92, M − 106, M − 158, 69, 43 (see structure *13*) and the silyl ether (*14*) at *m/e* 818 (M, corresponding to $C_{50}H_{70}(OSi(CH_3)_3)OCOCH_3$), M − 60, M − 92, M − 106, M − 131, 131 (base peak) and 43. The data discussed are hence in full agreement with the structure suggested for c.p. 473 (*12*).

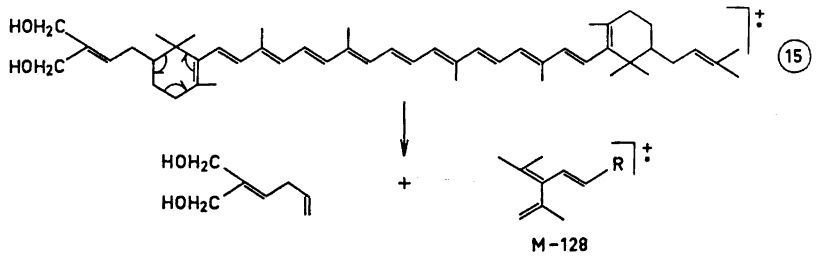
Attachment of the extra C_5H_8 unit at the 2-positions is preferred by analogy.[16,11] Losses of 96 mass units from a possible retro-Diels-Alder fragmentation of the β-ring were not observed for *12*, *13*, or *14*. Such losses are not obtained for unsubstituted β-rings and are apparently restricted to β-rings with oxygenated alkyl substituents; see below. An alternative attachment of the extra C_5H_8 unit to 3- or 4-position of the β-ring cannot be ruled out from the NMR evidence, the quality of the spectrum not permitting identification of the methylene protons of the β-ring. Since an acetoxy group in 3-position

also results in different shielding of the two *gem.* methyl groups of a β-ring,[16] the observed magnetic non-equivalence is no proof for a neighbouring asymmetric carbon atom, that is substitution in 2-position. However, the chemical shift positions suggest identical substitution pattern to c.p. 450 (*15*) discussed below.

The stereochemistry of the hydroxylated isopropylidene end-group is not experimentally established and arbitrarily chosen as *trans*, see Ref. 19.

C. p. 4 5 0 (*1 5*)

This carotenoid has a β-carotene-type absorption spectrum and R_F-value intermediate between isozeaxanthin and zeaxanthin. Judged by the melting point (168 – 171°C) and extinction coefficient ($\varepsilon = 137\,000$ compared with $\varepsilon = 130\,000$ [20] for β-carotene at 452 nm in acetone) this carotenoid was obtained in a pure state, yield *ca.* 3 mg. C.p. 450 (*15*) gave a mono (*16*) and a diacetate (*17*) on acetylation and a mono (*18*) and di(trimethylsilyl) ether (*19*) on silylation. IR data (Fig. 1) suggested that the hydroxy groups were primary and allylic (1005 cm^{-1}). However, the position allylic to the polyene chain was disregarded because of lacking reactivity with *p*-chloranil [21] and in allylic dehydration with acidified chloroform.[12] Further confirmation of the allylic, primary character followed from NMR evidence (singlet at τ 5.97, 4 H). Combination of NMR and mass-spectrometric data lead to structure *15* for c.p. 450.



NMR signals at τ 8.03 (four in-chain methyl) and 8.31 (two end-of-chain plus one extra methyl) confirmed the β-carotene-type chromophore. The third methyl group at resonance at τ 8.31 together with a signal at τ 8.39 (one methyl) was attributed to an isopropylidene group. Methyl singlets at τ 8.92 and 9.06 (two plus two methyl groups) were ascribed to two identical pairs of magnetically non-equivalent *gem.* dimethyl groups. Since attachment of the hydroxylated C_5-unit to 2- (or 3-) positions follows from the M – 128 ion in the mass spectrum of c.p. 450 (*15*) (shifted to M – 212 in the diacetate (*17*)), ascribed to a retro-Diels-Alder fragmentation of the β-ring, identity in chemical shift position of the two pairs of *gem.* dimethyl groups suggests that the unhydroxylated C_5-unit is also attached to 2′- (or 3′-) position. Differentiation between 2- and 3-substitution on the basis of the multiplicity of the allylic methylene (C – 4) signal is not possible on a 60 MC instrument – the

signal being too close to the in-chain methyl signals. 2-Substitution is preferred by analogy with the aliphatic representatives studied here and from biosynthetic considerations.[16,11]

The mass spectrum of c.p. 450 (*15*) had its molecular ion at *m/e* 704 (corresponding to $C_{50}H_{70}(OH)_2$), confirmed by M−92, M−106 and M−158 peaks. Losses of 18 and 128 mass units discussed above were of further diagnostic value. The diacetate (*17*) had characteristic peaks at *m/e* 788 (M), M−92, M−106, M−158 and peaks ascribed to the oxygenated end group at M−43, M−60, M−60−60, and M−212 (discussed above). The mass spectrum of the di(trimethylsilyl) ether (*19*) was in further agreement.

A chemical proof for the β-glycol arrangement in c.p. 450 (*15*) was sought by reaction with benzaldehyde.[22] No condensation product was obtained under conditions where the carotenoid survived. However, the data obtained are in full accordance with structure *15* for c.p. 450, a carotenoid that may easily be mistaken for zeaxanthin if not satisfactorily characterized.

Table 2. Required eluents from alumina activity grade 3, R_F-values on kieselguhr paper (Schleicher & Schüll No. 287) and partition ratios of the *trans* compounds studied.

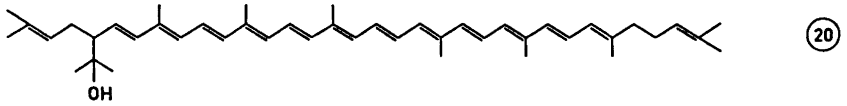
Number	Compound	Eluent	R_F-value				Partition ratio	
			0 %[a]	2 %	5 %	10 %	Pet.ether/ 95 % methanol	Pet.ether/ 85 % methanol
4	C.p. 496	60 % ether[b]				0.59	40:60	
5	mono-TMS ether			0.31	0.61			
6	di-TMS ether		0.81					
8	bis-anhydro			0.72			100:0	
9	C.p. 470	40 % ether				0.45	45:55	
11	di-TMS ether			0.68				
12	C.p. 473	70 % ether			0.25	0.79	32:68	94:6
13	acetate				0.60		74:26	
14	acetate TMS ether		0.25	0.78				
15	C.p. 450	100 % ether			0.28	0.66	27:73	80:20
16	monoacetate			0.25	0.58			
17	diacetate			0.63	0.99		100:0	
19	di-TMS ether		0.98					
20	C.p. 482	20 % ether			0.54		79:21	
21	TMS ether			0.75				
22	Lycopene	0 % ether	0.33	0.63			100:0	
23	C.p. 450-mono-ol	30 % ether		0.30	0.58		74:26	
24	acetate			0.35				
25	C.p. 473-mono-ol	30 % ether			0.57			
26	TMS ether		0.26				75:25	
27	C.p. 435	5 % ether	0.40	0.81			100:0	

[a] Acetone in petroleum ether.
[b] In petroleum ether.
TMS: Trimethylsilyl.

Awaiting nomenclature rules for C_{50}-carotenoids we have desisted from creating a trivial name for this new carotenoid, which is regarded as a 2-(dihydroxy-isopentenyl)-2′-isopentenyl-β-carotene. Lack of material prevented ORD measurements in attempts to investigate the stereochemistry of the asymmetric carbon atoms in c.p. 450 as well as in the other new carotenoids studied.

C. p. 4 8 2 = 2 - i s o p e n t e n y l - 3, 4 - d e h y d r o - r h o d o p i n (*2 0*)

Arguments in favour of structure *20* for this first C_{45}-carotenoid have been presented separately,[10] and experimental details are included in this paper. The mass spectrum of the trimethylsilyl ether (*21*) agreed with the previous assignment. *21* had molecular ion *m/e* 692 (corresponding to $C_{45}H_{63}OSi(CH_3)_3$). M − 131 and *m/e* 131 (base peak) ions confirmed the hydroxylated end-group, *cf.* structure *11*.



(20)

L y c o p e n e (*2 2*)

One C_{40}-carotenoid was shown to be present. Crystalline lycopene was identified by visible, IR, NMR and mass-spectral data. A minor pigment F of Starr and Saperstein [4] was suspected to be lycopene.

M i n o r u n i d e n t i f i e d c a r o t e n o i d s

Three minor carotenoids were not obtained in the pure state and only partly characterized.

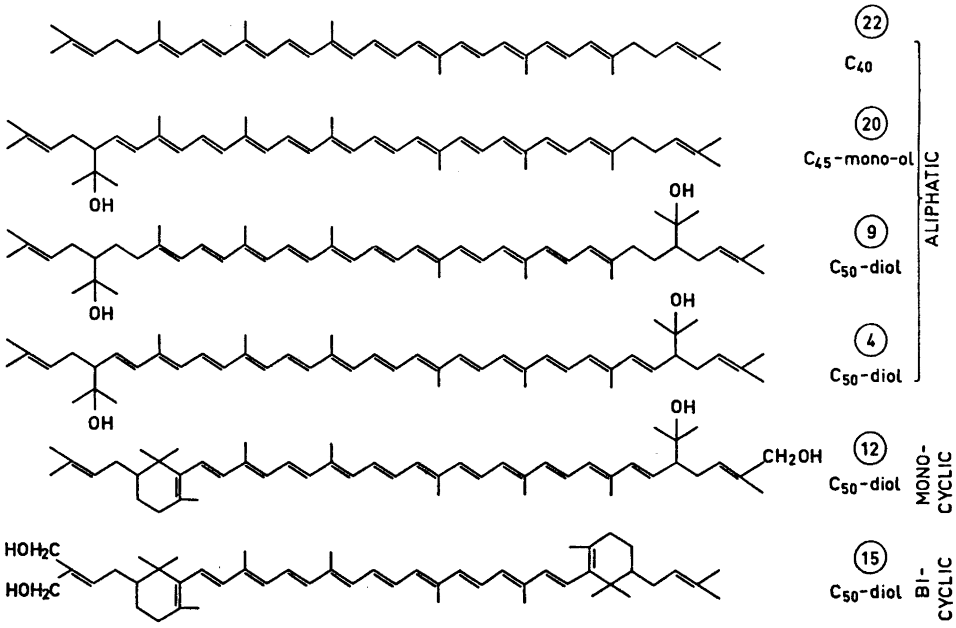
C.p. 450-mono-ol (*23*), probably the carotenoid previously identified as cryptoxanthin (*3*),[4] had a β-carotene-type absorption spectrum and gave a monoacetate (*24*) on acetylation. The acetate (*24*) furnished no silyl ether on silylation. The partition ratio and R_F-value indicate a C_{50}-mono-ol analogous to c.p. 450 (*15*) or possibly a C_{45}-mono-ol. Its mass spectrum could not be obtained.

C.p. 473-mono-ol (*25*) exhibited absorption spectrum corresponding to c.p. 473 (*12*). No acetate was formed on acetylation and a mono(trimethylsilyl) ether (26) on silylation. The polarity data (Table 2) suggest a tertiary C_{50}-mono-ol analogous to c.p. 473 (*12*) or possibly a C_{45}-mono-ol. The mass spectrum could not be obtained.

C.p. 435 (*27*) is presumably identical with Pigment H of Starr and Saperstein.[4] This pigment had an absorption spectrum more resembling that of neurosporene (aliphatic nonaene) than β-zeacarotene (monocyclic nonaene). It was more strongly adsorbed than neurosporene. Hydroxyl groups appear to be absent since it gave no acetate or silyl ether. Treatment with acidified chloroform provided a product with prolonged chromophore. Ether-oxygen may be present.

GENERAL CONCLUSION

The carotenoids encountered in *Corynebacterium poinsettiae* are compiled below. The distribution pattern, comprising C_{40}-, C_{45}-, and C_{50}-carotenoids at various oxygenation levels lends support to the hypothesis previously advanced for the biosynthesis of C_{50}-carotenoids.[16,11,23]



Carotenoids more polar than c.p. 496 (*9*) like c.p. 473 (*12*) and c.p. 450 (*15*) were not reported by Starr and Saperstein.[4] Incomplete extraction in their case or more likely stronger aeration in our experiments may account for this deviation in results. The identity of the pigments referred to as lycoxanthin and cryptoxanthin by Kunisawa and Stanier [7] is not clear; *cf.* Lot 1 (their medium) Table 1.

The finding by Kunisawa and Stanier [7] of phytoene and phytofluene in *Corynebacterium poinsettiae* under particular conditions has some bearing on the early steps of C_{50}-carotenoid biosynthesis, and agree with the more recent results by Weeks and co-workers [24] on the biosynthesis of C_{50}-carotenoids in *Flavobacterium dehydrogenans*.

The suggestion by Starr and Saperstein [4] that thiamine effects the cyclization of carotenoids in *Corynebacterium poinsettiae* is still valid also on the basis of the revised structures.

EXPERIMENTAL

Biological material. Corynebacterium poinsettiae Starr and Pirone was obtained from Professor R. Y. Stanier, Department of Bacteriology, University of California, Berkeley.

Cultivation. In introductory experiments cultures (100 ml) were grown as described by Kunisawa and Stanier [7] and analysed after 3 days of growth. For Lot 1 their medium B was used. Lots 2, 3, and 4 were grown in the medium described by Starr and Saperstein [4] with 1, 10, and 100 μg thiamine/l culture, respectively.

The mass cultures were grown in the latter medium in 170 l batches with strong aeration at 30°C, pH 6.6, in a fermentor described elsewhere [25] using 5 % inoculum. For batches 1, 2, and 3 10 μg thiamine/l culture and for batch 4 1000 μg thiamine/l culture were employed. The cultures entered the stationary phase of growth after 22—36 h, at which stage batch 1 was harvested. However, carotenoid synthesis proceeded in the stationary growth phase and the other batches were harvested by centrifugation 6—14 h after this phase was reached. The cells were stored frozen.

Materials and methods. When not specified these were as described elsewhere.[26] IR spectra (in KBr) were recorded on a Perkin Elmer Hitachi Model 137 grating spectrometer, NMR spectra (in $CDCl_3$) on a Varian A—60 A instrument and mass spectra on an MS 902 mass spectrometer with direct inlet system at 70 eV, *ca.* 200°C. Partition ratios of the carotenoids studied are compiled in Table 2 without further reference.

Pigment extraction. Lot 1 was extracted fairly completely according to the method of Kunisawa and Stanier,[7] Lots 2—4 and Batch 1 less satisfactorily with acetone in a cell homogenizer using ballotini beads and CO_2-ice. The combined batches 2+3+4 (1950 g wet weight) were treated with lysozyme (800 mg) in 0.5 M phosphate buffer pH 7.7 (1.5 l) for 20 h at 30°C. The resulting foamy cell suspension (6 l) was extracted with acetone (CO_2-ice) in a Waring blender and by standing overnight (4 times), thereafter in the same manner with methanol (4 times). In this case pigment extraction was complete, yield 0.02 % carotenoids of the extracted residue.

Saponification. Introductory experiments revealed that the pigments were not altered by alkali treatment, and saponification under standard conditions was employed in order to remove oily contaminants.

Chromatography and carotenoid composition. Column chromatography was performed on Spence type H alumina, activity grade 3; paper chromatography was carried out by the usual method. The carotenoid yield and the composition of the various cultures are compiled in Table 1. The individual components were repeatedly chromatographed on alumina columns (see Table 2) until apparently homogeneous.

C. p. 496 = bisanhydrobacterioruberin (*4*)

Characterization. 4 was crystallized and recrystallized from acetone-petroleum ether, yield *ca.* 6 mg, m.p. 170—171°C. For the crystalline *trans* isomer abs. max. in petroleum ether were located at 368, 387, 465, 493, and 527 nm and in acetone at (390), 471, 498 (ε=182 000), and 533 nm. The spectral shape corresponded to that of spirilloxanthin.[27] Iodine catalysed photochemical isomerization gave a neo A (abs. max. (389), 470, 492, and 527 nm in acetone, 23 % of total carotenoid), neo B (370, 388, 464, 490, and 522 nm, 17 % of total) and neo C (370, 389, (467), 490, and (522) nm, 6 % of total) isomer with R_F=0.60, 0.68, and 0.79, respectively on kieselguhr paper (10 % acetone in petroleum ether). The IR spectrum of *trans 4* (Fig. 1) had (only diagnostically useful frequencies are cited here and in the following) ν 3400, 1160, 905 (*tert.* OH), and 965 (*trans* disubst. double bonds) cm^{-1}. The NMR spectrum ($CDCl_3$) had methyl signals at τ 8.02 (four in-chain methyl), 8.07 (two in-chain/end-of-chain methyl), 8.33 and 8.39 (four isopropylidene methyl), 8.81 and 8.83 (four methyl at *tert.* OH), and allylic methylene at τ 7.85 (*ca.* 4H). The mass spectrum had peaks in the upper mass region at *m/e* 704 (M), M—18, M—18—18, M—58, M—76, M—87, M—92, M—106, M—58—58, M—106—18, M—58—69, M—106—18—18, M—87—58, M—158, and M—106—58.

In co-chromatography tests on kieselguhr paper with the stereoisomeric set of bisanhydrobacterioruberin from *Halobacterium salinarium* [11] no separation of the corresponding isomers of the two sets was obtained.

4 (0.1 mg aliquots) gave no acetate on standard acetylation, no reduction product with lithium aluminium hydride and no product with extended chromophore on treatment with acidified chloroform.[28]

Silylation. In standard silylation [11] at —35°C *4* (0.5 mg) was completely reacted after 10 min. The mono(trimethylsilyl) ether (*5*) was present in maximum quantity

(40 % of total) after 1 min 40 sec and the di(trimethylsilyl) ether (*6*) was quantitatively formed after 30 min. The diether (*6*) was hydrolysed [29] to *4* with alkali.

The stereoisomeric set of the diether (*6*) was not separated from that of bisanhydrobacterioruberin di(trimethylsilyl) ether of *Halobacterium salinarium* origin on kieselguhr or aluminium oxide papers.

Dehydration. *4* (4.7 mg) in dry pyridine (2 ml) was dehydrated with phosphorus oxychloride (0.1 ml) at 50°C for 1 h; pigment recovery was 40 %. Other experiments gave a similar result. The monoanhydro (*7*) and bisanhydro (*8*) products exhibited the same absorption spectrum in visible light as *4*.

The bisanhydro derivative (*8*) had ν_{max} 960 (*trans* disubst. double bonds) and 882 cm^{-1} (medium intensity, terminal methylene). *8* was less strongly adsorbed than 3,4-dehydro-lycopene. Treatment of *8* with 10 % KOH-methanol for 10 h or with saturated ethanolic sodium ethoxide in ethanol (1:1) for 19 h gave no products with prolonged chromophore.

C. p. 470 = 3, 4, 3′, 4′-tetrahydrobacterioruberin (*9*)

Characterization. A fraction containing *9* (0.5 mg) was obtained, abs. max. 345, 362, 448, 473, and 504 nm in acetone. The mass spectrum had peaks in the upper mass region at *m/e* 708 (M), M−2, M−92, and M−106, and at *m/e* 69.

9 (0.4 mg) gave no acetate on acetylation.

Silylation. *9* (0.2 mg) was silylated at −35°C. The formation of an intermediary mono (trimethylsilyl) ether (*10*) was observed by paper chromatography. The ultimate di-(trimethylsilyl) ether (*11*) had characteristic peaks in its mass spectrum at *m/e* 852 (M), M−2, M−90, M−92, M−106, 131 (base peak).

C. p. 473 (*12*)

Characterization. *12*, yield *ca.* 4 mg, had m.p. 110°C after recrystallization from acetone-methanol. *trans* *12* had abs. max. in petroleum ether at 362, 446, 473, and 504 nm, in acetone at (365), 451, 478 (ε=104 000), and 509 nm, and in chloroform at (371), (460), 486, and 520 nm. The spectra corresponded to those of saproxanthin.[30] On iodine catalysed isomerization a neo A isomer was produced (abs. max. (350), 365, (448), 471, and 501 nm, 30 % of total mixture, R_F=0.71 on kieselguhr paper with 10 % acetone in petroleum ether). The IR spectrum (Fig. 1) of crystalline *12* had ν_{max} 3400 (OH), 1160 and 905 (*tert.* OH), 1005 (*prim.* allylic OH) and 965 (*trans* disubst. double bonds) cm^{-1}. The NMR spectrum ($CDCl_3$) had signals at τ 5.96 (=C(OH)−CH_2OH, 2 H), 8.02 (four in-chain methyl), 8.08 (one in-chain/end-of-chain methyl), 8.30, 8.32, 8.39 (four *tert.* methyl), 8.78 (two *gem.* methyl attached to *tert.* OH) and 8.92, 9.05 (two *gem.* methyl). The mass spectrum had peaks in the upper mass region at *m/e* 704 (M), M−18, M−58, M−69, M−87, M−92, M−106, M−158, M−182 (*m/e* 522.4239; calc. 522.4225 for $C_{39}H_{54}$).

In co-chromatography tests *12* was more strongly adsorbed than saproxanthin [30] on kieselguhr paper.

No reduction product of *12* was obtained with lithium aluminium hydride, and no product with prolonged chromophore on treatment with acidified chloroform.[28]

Acetylation. *12* (0.8 mg) was acetylated in the usual manner. No intermediate was observed during reaction to the monoacetate (*13*), which was quantitatively formed after 70 min at room temperature; pigment recovery was 100 %. The absorption spectrum of *13* corresponded to that of *12*. The mass spectrum of *13* had peaks in the upper mass region at *m/e* 746 (M), M−18, M−42, M−58, M−69, M−92, M−106 and M−158.

Silylation. The acetate (*13*, 0.6 mg) was silylated at room temperature. The single product (*14*) had abs. max. corresponding to *12* and its mass spectrum peaks at *m/e* 818 (M), M−42, M−72, M−106, M−131, and M−158, 131 (base peak). On alkaline hydrolysis [29] *14* was converted quantitatively to *12* during 18 h.

C. p. 450 (*15*)

Characterization. 15, crystallized from acetone-petroleum ether, yield *ca.* 4 mg, had m.p. 168–171°C and abs. max. in petroleum ether at (425), 451, and 480 nm, in acetone at (427), 454 (ε=137 000, reported for synthetic β-carotene 137 000 in petroleum ether,[31] found 129 000 in acetone) and 481 nm in methanol at (420), 448, and 473 nm. Iodine catalysed isomerization in benzene solution resulted in a 16 % drop in extinction coefficient and a neo A isomer was produced (abs. max. (430), 452, and (480) nm in acetone, R_F=0.86 on kieselguhr paper with 10 % acetone in petroleum ether). The IR spectrum (Fig. 1) of crystalline *15* had ν_{max} 3400, 1005 (*prim.* allylic OH), and 968 (*trans* disubst. double bonds) cm^{-1}. The NMR spectrum had signals at τ 5.97 (=C(H)–CH_2OH, 2 H), 8.02 (four in-chain methyl), 8.32, 8.39 (four *tert.* methyl), 8.92 and 9.06 (two *gem.* methyl).

On aluminium oxide paper (20 % acetone in petroleum ether) the following R_F-values were determined in co-chromatography tests: zeaxanthin 0.22, *15* 0.35, and isozeaxanthin 0.39.

No product with prolonged chromophore was obtained on treatment of *15* (0.1 mg) with acidified chloroform.[28] Oxidation with *p*-chloranil was negative.

15 (0.6 mg) in benzaldehyde (0.5 ml) was kept at 40°C for 20 h. No condensation product was revealed by paper chromatography. On further standing at 80°C *15* decomposed.

Acetylation. 15 (0.3 mg) was acetylated. After 18 min at room temperature *15* was completely reacted. A monoacetate (*16*) was present in maximum amount (45 % of total) after 11 min and the diacetate (*17*) was quantitatively formed after 100 min. The diacetate (*17*) had *m/e* 788 (M), M–15, M–43, M–60, M–92, M–106, M–60–60, M–69–60, M–130, M–158, and M–212. The diacetate (*17*, 0.1 mg aliquots) gave no silyl ether on silylation, and was converted to *15* on treatment with lithium aluminium hydride.

Silylation. 16 (0.2 mg) gave a final di(trimethylsilyl) ether (*18*) on silylation. *18* had *m/e* 848 (M), M–73, M–90, M–106, M–158, no peak at M–131, low peak at *m/e* 131.

C. p. 482 (*20*)

Characterization. 20, crystallized from acetone-petroleum ether, yield ca. 4 mg, had m.p. 151–153°C, abs. max. in petroleum ether at 356, 374, 454, 482, and 514 nm, % III/II=76, in acetone at (360), 377, 458, 486 (ε=172 500 reported 195 000 for 3,4-dehydro-rhodopin [32]), and 518 nm, % III/II=79, and in benzene at (365), 385, 470, 499, and 533 nm, % III/II=71. The spectra corresponded to those of rhodovibrin.[25] On iodine catalyzed isomerization in benzene a 29 % drop in extinction value and a hypsochromic shift of 5 nm resulted. A neo A isomer (abs. max. 375, 462, 482, and 512 nm in acetone, 28 % of total) and a neo B isomer (abs. max. 375, 460, 474, and 507 nm, 14 % of total) with R_F-values 0.60 and 0.72, respectively, on kieselguhr paper (5 % acetone in petroleum ether) were produced. The IR spectrum (Fig. 1) had ν_{max} 3400, 1160, and 910 (*tert.* OH), and 960 (*trans* disubst. double bonds) cm^{-1}. The NMR spectrum exhibited signals at τ 3.2–4.5 (olefinic protons of the polyene chain), 4.9 (2 H olefinic, isopropylidene), 7.9 (*ca.* 6 H allylic methylene), 8.02 (four in-chain methyl), 8.08 (one in-chain/end-of-chain methyl), 8.18 (one end-of-chain methyl), 8.32, 8.39 (four isopropylidene methyl) and 8.79, 8.81 (two methyl at *tert.* OH). The mass spectrum had peaks in the upper mass region at *m/e* 620 (M), M–18, M–58, M–69, M–92, M–106, and M–106–18.

20 (0.4 mg) gave no acetate on standard acetylation; pigment recovery was 90 %.

Silylation. 20 (0.4 mg) was silylated at –35°C. A single product (*21*) was formed in quantitative yield after 30 min; pigment recovery was 94 %. The product (*21*) had peaks in the upper mass region at *m/e* 692 (M), M–90, M–106, M–131, base peak at *m/e* 131.

Lycopene (*21*)

After crystallization from acetone-petroleum ether *21*, yield *ca.* 3 mg, had m.p. 148–150°C (reported 172–173°C for synthetic lycopene [31]), abs. max. in petroleum ether at (345), (361), 444, 472 (ε=134 000, reported 186 000 [33] for pure lycopene) and 503 nm, % III/II=78, ν_{max} 955 cm^{-1}. τ 7.85 (*ca.* 8 H), 8.0 (four methyl), 8.17 (two methyl), 8.32 and 8.39 (four methyl), *m/e* 536 (M), M−69, M−92, M−106. No acetate was obtained on acetylation and no silyl ether on silylation of aliquots (0.3 mg). The stereoisomeric set of the present compound was identical in co-chromatography tests on kieselguhr paper with that of authentic lycopene.

Minor carotenoids

C. p. 450-mono-ol (23). Trans 23 had abs. max. in acetone at 434, 455, and 484 nm. The spectrum corresponded to that of β-carotene.[20] Acetylation of *23* (0.1 mg) gave a monoacetate (*24*). No reaction intermediates were observed by paper chromatography. The monoacetate (*24*) gave no silyl ether on silylation.

C. p. 473-mono-ol (25). Trans 25 had abs. max. in acetone at 450, 475, and 507 nm. The spectrum corresponded to that of *12*. Acetylation of *25* (0.2 mg) gave no acetate; pigment recovery was 95 %. *25* (0.2 mg) gave a mono(trimethylsilyl) ether (*26*) on silylation (no reaction intermediates).

C. p. 435 (27) had abs. max. in petroleum ether at 410, 435, and 463 nm, % III/II=56, and in acetone at 414, 439, and 568 nm, % III/II=52. In co-chromatography tests the following R_F-values were established on kieselguhr paper (petroleum ether): β-zeacarotene 0.93, *27* 0.66, chlorobactene 0.48, and on aluminium oxide paper (1 % acetone in petroleum ether) neurosporene 0.74 and *27* 0.47.

27 (0.1 mg) gave no acetate on acetylation; pigment recovery was 100 %, neither did *27* (0.1 mg) give any silyl ether on silylation; pigment recovery was 81 %. Treatment of *27* (0.01 mg) with lithium aluminium hydride in ether gave 65 % pigment recovery (unreacted *27* only). Treatment of *27* (0.01 mg) with 0.03 N HCl in chloroform [28] for 1 min resulted in a bathochromic shift. The products were chromatographed on aluminium oxide paper. The main product was somewhat more polar than lycopene and had abs. max. at 450, 475, and 507 nm, % III/II=30 in acetone.

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