Carotenoid Glycoside Esters from the Thermophilic Bacterium *Meiothermus ruber*

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The thermophilic bacterium *Meiothermus ruber* produces a series of carotenoid glycoside esters. The major carotenoid has been identified as 1′-β-glucopyranosyl-3,4,3′,4′-tetradehydro-1′,2′-dihydro-β,ψ-caroten-2-one (**1**). It is acylated at the 6″-position of the glucose unit by a series of C_{10}−C_{17} fatty acids. The structure of **1** was established by spectral means, including complete assignment of the ^{1}H and ^{13}C NMR resonances by inverse 2D NMR spectroscopy. These carotenoids are thought to play roles in stabilizing membranes of this thermophilic organism.

Meiothermus ruber (Loginova, Egorova, Golovacheva, and Seregina 1984) comb. Nov. (phylum *Bacteria*) (previously *Thermus ruber*) is a red-pigmented thermophile, widely distributed in hot springs and artificial thermal environments. It has an optimal growth temperature of 60 °C.[1,2] *M. ruber* has been previously reported to produce bright red and occasionally orange intracellular pigments, with absorption spectra similar to retro-dehydro-γ-carotene.[3] The end products of the carotenoid biosynthetic pathway of the related thermophilic bacterium, *Thermus thermophilus*, are zeaxanthin glycoside esters.[4] *Thermus aquaticus* and *Thermus flavus* also produce bright orange pigments that are regarded as α-carotene-like.[5,6]

Glycosides of C_{40} carotenoids are found in some bacteria and algae. However, their fatty acid monoesters are less common, being found in only a few species of bacteria, namely, *T. thermophilus*,[4,7] *Rhodococcus rhodochrous*,[8] *Myxococcus species*,[9] and in the photosynthetic filamentous bacteria, *Chloroflexus auratiacus* and *Chloroflexus aggregans*.[10]

Thermophilic bacteria are thought to possess special mechanisms for membrane stabilization that allow growth at high temperatures. Carotenoid production may be one such mechanism, as it has been reported that membrane stabilization is a key biological function of bacterial carotenoids.[11] This is based on the fact that the lengths of carotenoid molecules are similar to the depth of a lipid bilayer. It has been suggested that carotenoid glycoside esters may span the lipid bilayer and act as stabilizers of the membranes of thermophiles at high temperature.[4] Carotenoid glucoside esters *of M. ruber* may act in a similar way.

In the present study, 1′-β-glucopyranosyl-3,4,3′,4′-tetradehydro-1′,2′-dihydro-β,ψ-caroten-2-one (**1**) has been identified as the major pigment in *M. ruber*. This carotenoid exists as a series of acylated derivatives with C-10 to C-17 straight and branched-chain fatty acids esterified to the 6 position of the sugar (Chart 1).

Results and Discussion

Compound **1** was isolated as an amorphous solid. MALDI-MS gave a molecular ion at *m*/*z* 726.83 corresponding to a molecular formula of $C_{46}H_{62}O_7$, which was confirmed by observation of $[M + H]^+$ by high-resolution electrospray MS (found *m*/*z* 727.4552, calcd 727.4573). The UV/vis spectrum of **1** in EtOH exhibited a broad maximum at 478 nm with poorly defined shoulders at 450 and 506 nm. On addition of $NaBH_4$, the maximum underwent a hypsochromic shift to 473 nm and exhibited an increase in vibrational fine structure. This indicated the presence of a conjugated aldehyde or ketone group. This hypsochromic shift is small and ruled out a conjugated ketone at the 4-position of a cyclic carotenoid end-group but suggested that there may be a conjugated ketone at the 2-position. A progressive change in absorption pattern was observed over a 25-min period. This supported the presence of a conjugated ketone functional group, as aldehydes are generally reduced completely within 30 s, while complete reduction of a ketone occurs over a longer period.[12] The IR spectrum showed a weak band at 1650 cm^{-1}, indicative of a hindered conjugated ketone.

1D ^{1}H NMR and 2D ^{1}H−^{1}H COSY, ^{1}H−^{13}C HSQC and ^{1}H−^{13}C HMBC NMR experiments were performed on approximately 250 μg purified pigment, post-saponification. The ^{1}H NMR spectrum of the molecule was consistent with a carotenoid glycoside. Crowded methyl and olefinic regions were observed at 1.2−2.1 ppm and 5.8−6.9 ppm, respectively, with a range between 3.1 and 4.5 ppm being characteristic of a sugar functional group. The relatively complex ^{1}H NMR spectrum, compared to the spectra of symmetrical carotenoids, demonstrated that the compound lacked symmetry and that it possessed different functionalities at the end groups. Proton chemical-shift assignments were made through interpretation of the 1D ^{1}H NMR spectrum and a ^{1}H−^{1}H correlated COSY spectrum. The COSY experiment showed connectivities between protons within each isolated spin system, and coupling constants were calculated from the 1D ^{1}H NMR spectrum. A total of eight isolated spin systems were observed.

As a generalization, from numerous reports of carotenoid NMR spectra in the literature, vicinal coupling constants across trans double bonds have values between 13.5 and 16.8 Hz and tend to decrease in size toward the central part of the conjugated chain. In contrast, single-bond couplings in polyenes usually fall in the range of 10.5 to 12.0 Hz and tend to increase in value toward the center of the molecule.[13] Olefinic protons were observed in the range of 5.8 to 7.0 ppm in the 1D ^{1}H NMR spectrum and exhibited

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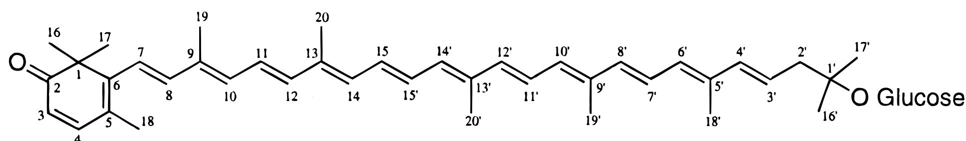
Chart 1



trans-couplings in the range of 11.3 to 15.8 Hz. Signals in the olefinic region were crowded and observed as broad multiplets due to long-range couplings to allylic methyl groups. As a result, some spin couplings could not be measured. One four-spin system was observed and assigned to the central region of the conjugated polyene chain. COSY cross peaks were observed from H-14 to H-15, H-15 to H-15′, and H15′ to H-14′. The coincidental proton chemical shifts (6.65 ppm) of H-15 and H-15′ were in agreement with literature value of 6.6 ppm.[13] Three three-spin systems were attributed to other parts of the central polyene chain region of the carotenoid by comparison of chemical shift values and coupling constants to literature values.[13] ^{1}H NMR chemical shifts of the central part of the conjugated chain were least influenced by end-group functions. H-14 and H-14′ gave rise to coincidental chemical shifts at 6.35 ppm, and H-15/H-15′ and H-20/H-20′ gave rise to coincidental shifts of 6.65 and 1.98 ppm, respectively. These values are consistent with literature values of the central region of all C_{40} carotenoids for which proton NMR data have been reported.[13] H-11 gave rise to a doublet of doublets that exhibited cross peaks to doublets arising from H-10 and H-12. A coupling constant of 11.03 Hz was measured for H-10 to H-11. However, due to crowding in the olefinic region, the coupling constant for H-11 to H-12 could not be determined. H-7′ gave rise to a doublet of doublets in the 1D ^{1}H NMR spectrum, that showed COSY cross peaks to H-6′ and H-8′, with trans coupling constants of 11.6 and 14.8 Hz, respectively. Similarly, a three-spin system was observed for H-10′−H-11′−H-12′. A doublet of doublets was observed for H-11′ in the 1D ^{1}H NMR spectrum. H-11′ exhibited trans coupling to H-10′ (11.4 Hz) and to H-12′ (14.9 Hz), observed as cross peaks in the COSY spectrum.

A two-spin system was observed as a single cross peak in the COSY spectrum between the olefinic protons at 6.39 and 6.58 ppm, which were assigned as H-7 and H-8, respectively. A trans coupling constant of 15.8 Hz was measured from the 1D ^{1}H NMR spectrum. This was the largest coupling observed and was in agreement with literature estimates between 15.2 and 16.8 Hz.[13] A four-spin system, H_2-2′−H-3′−H-4′ was also observed. H-3′ gave rise to a doublet of triplets in the 1D ^{1}H NMR spectrum, suggesting coupling to methylene and methine protons. H-3′ exhibited a COSY cross peak to H-4′ in the olefinic region, with a trans coupling of 15.4 Hz. A cross peak was also observed between H-3′ and H_2-2′, in the methylene region (2.42 ppm, 7.4 Hz).

The 'sugar' region of the 1D ^{1}H NMR spectrum was relatively free from crowding, and the resonances were readily assigned to a glucose moiety by interpretation of the ^{1}H−^{1}H correlation spectrum. Only one spin system was present, and this was traced from C-1″ through to C-6″ of the sugar. H-2″ gave rise to a doublet of doublets that exhibited couplings to a doublet of H-1″ (7.8 Hz) and to a doublet of doublets arising from H-3″ (9.0 Hz). H-3″ was also coupled to a doublet of doublets of H-4″ (9.0 Hz). In addition, H-4″ exhibited coupling to H-5″ (4.5 Hz). H-6$_a$″ and H-6$_b$″ were coupled to each other (11.8 Hz) and to H-5″ (2.4 and 4.4 Hz). H-5″ exhibited cross peaks to H-4″, H-6$_a$″ and H-6$_b$″. Due to the complexity of the multiplet shown by H-5″, coupling constants could not be measured directly. However, they have been obtained from splittings observed for the C-6″ protons and H-4″. ^{13}C NMR resonances confirmed that **1** is a *β*-glucoside (Table 1). In particular, the anomeric resonance at 98.7 ppm, together with a J_{H1-H2} coupling constant of 7.8 Hz, is consistent only with a *β*-glucopyranoside.[14,15]

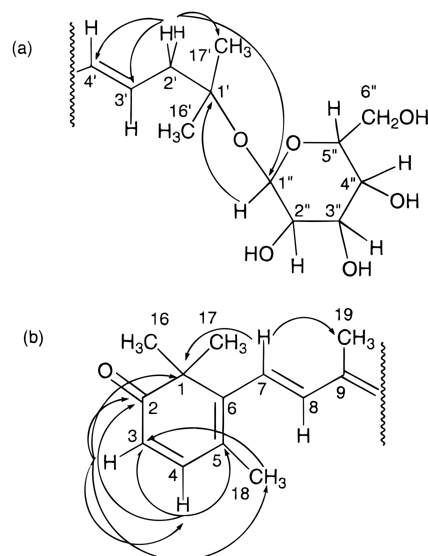


Figure 1. HMBC connectivities essential to structure determination of (a) the glucose moiety, and (b) the cyclic end-group of 1′-*β*-glucopyranosyl-3,4,3′,4′-tetrahydro-1′,2′-dihydro-*β*,*ψ*-caroten-1-one.

The cyclic carotenoid end-group of **1** showed little information in the COSY spectrum. One cross peak was observed for H-3 to H-4. The proton chemical shifts, 6.19 and 6.98 Hz, respectively, and a 9.9-Hz coupling constant were indicative of *cis*-olefinic protons, and the chemical shifts are typical of an α,*β*-unsaturated ketone in a six-membered ring.[13] The methyl region of the 1D ^{1}H NMR spectrum was crowded, and no COSY cross peaks were observed for these protons, due to the absence of protons on adjacent carbon atoms. Hence, confirmation of proton chemical shift assignments of the tertiary methyl groups was achieved through interpretation of the HMBC experiment.

Carbon chemical shifts were acquired indirectly through inverse ^{1}H−^{13}C correlation experiments. As only a limited amount of sample was available, these heteronuclear inverse correlation experiments were run with gradient pulses to reduce phase cycling and to allow adequate signal-to-noise ratios to be obtained in reasonable time. Protonated carbon chemical shifts were assigned through interpretation of the ^{1}H−^{13}C correlated HSQC experiment, and chemical shift assignments of the 10 quaternary carbon atoms were made through interpretation of the HMBC experiment, which exhibited long-range ^{1}H−^{13}C couplings of up to four bonds. The long-range ^{1}H−^{13}C HMBC experiment provided evidence of connectivities between previ-

Table 1. NMR Assignments of 1′-β-Glucopyranosyl-3,4,3′,4′-tetradehydro-1′,2′-dihydro-β,ψ-caroten-2-one

position	^{13}C δ[a]	^{13}C δ[b]	assignment	^{1}H δ[c]	multiplicity	*J* (Hz)	HMBC[d]
1		39.5	qC				
2		206[e]	qC				
3	129.9		CH	6.19	d	9.9	C: 18
4	160.0		CH	6.98	d	9.9	C: 1, 2, 6, 18
5		124.5	qC				
6		149.0	qC				
7	136.4		CH	6.39	d	15.8	C: 1 (weak), 19
8	143.7		CH	6.58	d	15.8	C: 1, 19
9		135.1	qC				
10	133.6		CH	6.24	d	11.3	
11	131.0		CH	6.71	dd	n.d.[g]	
12	139.5		CH	6.40	d	n.d.[g]	
13		135.0	qC				
14	126.0		CH	6.35	m	n.d.[g]	C: 11 (weak)
15	139.2		CH	6.65	m	n.d.[g]	
16	27.0		CH_3	1.31	s		
17	27.0		CH_3	1.31	s		
18		25.4	CH_3	2.07	s		C: 3, 5
19	12.8		CH_3	2.01	s		C: 10 (weak)
20	12.7		CH_3	1.98	s		C: 11, 13
1′		77.2	qC				
2′	46.28		CH_2	2.42	d	7.4	C: 3′, 4′, 17′, 1″
3′	126.6		CH	5.88	dt	7.4	C: 1′
						15.4	C: 18′
4′	138.7		CH	6.17	d	15.4	C: 18′
5′		136.5	qC				
6′	131.6		CH	6.11	d	11.6	C: 18′
7′	125.7		CH	6.65	dd	11.6	
						14.8	
8′	135.1		CH	6.35	d	14.8	
9′		136.0	qC				
10′	135.1[f]		CH	6.32	d	11.4	
11′	131.5[f]		CH	6.72	dd	11.4	
						14.9	
12′	140.9[f]		CH	6.50	d	14.9	
13′		135.0	qC				
14′	126.0		CH	6.35	m	n.d.[g]	
15′	139.2		CH	6.65	m	n.d.[g]	
16′	27.1		CH_3	1.26	s		C: 17′
17′	27.1		CH_3	1.26	s		C: 16′
18′	13.0		CH_3	1.93	s		C: 4′, 5′, 6′
19′	13.2		CH_3	2.00	s		
20′	12.8		CH_3	1.98	s		C: 11′, 13′
1″	98.7		CH	4.52	d	7.8	C: 1′
2″	75.1		CHOH	3.15	dd	7.8	C: 3″
						9.0	
3″	78.1		CHOH	3.36	dd	9.0	C: 4″
						9.0	C: 2″
4″	71.5		CHOH	3.28	dd	9.0	C: 3″
						4.5	C: 5″
5″	77.4		CHOH	3.25	m		
6″	62.6		CH_2OH	3.65, 3.52	dd	2.4, 11.8	
					dd	4.4, 11.8	

[a] From HSQC. [b] From HMBC. [c] From 1D ^{1}H and HSQC. [d] Only HMBC connectivities essential to structural assignment have been tabulated. [e] Folded peak. [f] The assignments for 10′, 11′, and 12′ may be interchanged. [g] Coupling constant not determined because of overlapping multiplets.

ously assigned proton spin systems. Resonances for the cyclic carotenoid end-group were assigned largely by interpretation of the HMBC experiment. A limited number of protons in this region of the molecule resulted in observation of only one two-spin system in the COSY experiment. H-3 showed long-range HMBC connectivities to C-1, C-2, C-3, C-4, and C-18. H-3 exhibited long-range coupling to C-18, and the methyl protons attached to C-18 exhibited connectivities to C-3 and C-5. A limited number of HMBC connectivities essential to structure determination is shown in Figure 1.

Several long-range couplings were observed from protons of the polyene chain to carbons of the cyclic end-group and provided a link between the COSY spin systems that were previously assigned. H-7 exhibited coupling to C-1 andC-19, and H-8 was coupled to C-1 and C-19. An ether linkage from C-1″ of glucose to C-1′ of the acyclic carotenoid end-group was confirmed by a long-range coupling from H-1″ of glucose to C-1′. This was supported by the presence of a four-bond coupling of the proton at C-2′ to C-1″ of glucose. Figure 1 shows HMBC connectivities that were essential to the structure elucidation of **1**. HMBC connectivities were observed from H-4′ and H-6′ to C-18′, linking the two isolated COSY spin systems. Long-range coupling was also observed from protons attached at C-20 to C-11 and C-13, and the C-19 methyl protons showed coupling to C-9, C-10, and C-12. Coupling from H-7 to C-19 completed the confirmation of structure from the cyclic carotenoid end-group through to C-13. Little coupling was observed in the HMBC experiment for the central region of the conjugated polyene chain. The C-20′ methyl protons exhibited long-range coupling to C-11′ and C-13′. However, these couplings

Table 2. Fatty Acids from Acylated Carotenoid Glycosides of *M. ruber*

identity	retention time (min:sec)	$[M + H]^+$ (*m/z*)	molecular mass	% total fatty acids
$C_{10:1}$	4:55	187	186	36.6
$C_{11:1}$	6:07	201	200	2.6
C_{14}	5:44	243	242	0.7
C_{15} b[a]	7:04	257	256	9.1
C_{15} b[a]	7:13	257	256	0.5
C_{15}	7:57	257	256	1.7
$C_{16:1}$	10:31	269	268	1.1
C_{16}	11:24	271	270	13.5
C_{17}	14:52	285	284	1.3
$C_{18:1}$	16:59	297	296	7.7
$C_{18:1}$	21:40	297	296	3.2
C_{18}	24:07	299	298	3.1

[a] b = branched-chain.

did not provide a link between individual spin systems. Due to the carotenoid nature of the molecule, it was assumed that the four-spin system observed in the COSY experiment is located in the central region of the molecule. This is supported by coincidental chemical shifts of 14/14′ and 15/15′ due to their distance from asymmetrical end-groups of the molecule.

Twelve fatty acids from *M. ruber* carotenoids were characterized by GC−MS analysis of the corresponding fatty acid methyl esters by comparison of retention times and fragmentation patterns with a standard mixture of bacterial fatty acid esters (Table 2). The major component was a $C_{10:1}$ fatty acid methyl ester (37%). A range of other minor components was identified, ranging from 11 to 17 carbon atoms in length. Two branched-chain C_{15} fatty acid esters were also identified. The 1D ^1H NMR spectrum indicates that the fatty acids are attached to the 6″-position of the glucose, as the C-6″ protons resonated approximately 1 ppm downfield in the ^1H NMR spectrum of the non-saponified pigment. A similar substitution is found in the carotenoid glycosides of *T. thermophilus.*[4] The major fatty acids of total cellular fatty acids of *M. ruber* have been identified as branched-chain compounds, and 2-hydroxy fatty acids were produced in moderate amounts.[16]

The structures of the minor metabolites of *M.ruber* have not been determined. However, on the basis of visible spectral properties, chromatographic retention characteristics, and molecular masses, it is possible to suggest probable constitutions for some of these components. In particular, MALDI MS of some of the fractions obtained by HPLC separation after saponification showed M^+ ions at *m/z* 548.46, 548.42, 567.37, 728.48, as well as several other peaks with masses in the range *m/z* 726−728 and 748−750, respectively. Some of the compounds are geometrical isomers, which separate easily on reversed-phase HPLC and may well be artifacts. Compounds with masses in the range 726−750 amu are obviously glycosides with less unsaturation and/or one more oxygen functionality than **1**. Of the compounds with masses 548−568 amu, some are almost certainly biosynthetic precursors with less modification at either of the unsymmetrical ends of the molecule. The corresponding UV/vis spectra give indications of the chromophores present, but there were insufficient compounds available for structural characterization. Current work involves investigations of mutants blocked at various stages in the biosynthetic pathway, and these studies should clarify the structures of the minor components.

The occurrence of these highly unsymmetrical carotenoids, found mainly in bacteria that exist in extreme environments, raises interesting questions concerning the enzymes involved. Presumably these carotenoids are embedded across the plasma membrane, and models for this have been published.[11] The enzymes of the carotenogenic pathway after geranylgeraniol diphosphate synthase are all membrane bound. To have only asymmetric products there must be active sites for terminal modifications of the acyclic precursors on different proteins that have active sites located on or near the different faces of the plasma membrane. The possibility of a reorientation, by a flip-flop mechanism, of this type of ridged rod-shaped carotenoids spanning the membrane seems unlikely, because that should lead, at least, to some symmetrical modification. Current genetic and molecular biology studies may answer these questions.

The occurrence of acylated carotenoid glucosides in the thermophile, *M. ruber*, is consistent with the proposed role of bacterial carotenoid glycoside esters in membrane reinforcement at high temperature by spanning the lipid bilayer.[4]

Experimental Section

General Experimental Procedures. The IR spectrum of **1** was recorded using an ATI Mattson Genis Series FTIR spectrometer. UV/vis spectral data were recorded using a Beckman DU7500 diode array spectrophotometer. NMR spectra were acquired on 250 μg of **1** using a Bruker Avance series DMX-600 spectrometer [600 MHz (^1H), 151 MHz (^{13}C)] in CD_3OD solution and referenced from the solvent. The HRMS of **1** was obtained using a VG Autospec time-of-flight electrospray mass spectrometer. MALDI-MS was performed using a Finnigan MAT MALDI mass spectrometer and α-cyano-4-hydroxycinnamic acid as matrix. GC−EIMS spectra were obtained using a Finnigan MAT GCQ instrument.

Changes in visible absorption were observed after the addition of 0.5 mg $NaBH_4$ to **1** dissolved in 1 mL EtOH. $NaBH_4$ was added to the solution of **1** after a visible absorption spectrum was recorded. Spectra were then recorded 30 s, 2, 5, and 30 min after the addition of the reducing agent. The ^1H NMR spectrum of **1** was recorded with a 90° pulse of 3.6 μs, 6600 Hz spectral width, and a 1-s relaxation delay between pulses in a spinning 5-mm sample tube at 25 °C. All 2D NMR spectra were recorded without spinning. A double-quantum filtered COSY spectrum was acquired using the standard Bruker pulse program and a gradient ratio of 10:20. The spectral width in both F1 and F2 was 3820 Hz. A relaxation delay of 1.8 s was employed between pulses. Altogether, 512 data points were accumulated in F1, and 4096 data points were used in F2. The F1 dimension was zero filled to 1 K, and the matrix was processed with a phase-shifted sine-squared function in both dimensions. A heteronuclear single quantum correlation (HSQC) experiment employed coherence selection using shaped gradients.[17] Gradient ratio = 40:10:40:−10. The spectral width in F2 = 4430 Hz, in F1 = 30 180 Hz; 512 data points were accumulated in F1, and 4096 data points were used in F2. A relaxation delay of 2.4 s was employed between pulses. The F1 dimension was zero filled to 1 K, and the 2D matrix was processed with a $\pi/2$ phase-shifted sine-squared function in both dimensions. HMBC experiment was performed using an F2 spectral width of 4430 Hz and an F1 spectral width of 30 180 Hz. Coherence selection using shaped gradients was employed with a gradient ratio = 50:30:40. A relaxation delay of 2.0 s was used between pulses, with 512 data points being accumulated in F1 and 4096 data points acquired in F2. The F1 dimension was zero filled to 1 K, and the 2D matrix was processed with a phase-shifted sine-squared function in both dimensions.

Extraction and Isolation. *M. ruber* cultures were grown on solid medium at 60 °C. Cells (4.98 g fresh wt) were extracted using MeOH and a mixture of MeOH−CH_2Cl_2. The extract was evaporated to dryness, and the residue (0.24 g) was partitioned between CH_2Cl_2 and H_2O (1:1 v/v). The CH_2Cl_2 phase was evaporated to dryness, and the residue was saponified in 10%

KOH–MeOH at room temperature overnight. The carotenoid fraction obtained after saponification of the crude pigment extract of *M. ruber* was further purified by multiple injections onto HPLC. Preliminary HPLC separation of pigments was performed by isocratic elution using $MeOH-CH_3CN-H_2O$ (86:7:7 v/v/v) and a Waters Symmetry C_{18} column. This was followed by isocratic elution using $MeOH-H_2O$ (19:1 v/v) and an Activon GoldPak C_{18} column.

A fatty acid fraction obtained from saponification of a carotenoid extract of *M. ruber* was partitioned between alkali solution (pH 9) and Et_2O to remove basic and neutral components. The aqueous phase, containing fatty acids, was acidified to pH 2 and extracted with Et_2O. The fatty acid fraction was methylated using CH_2N_2. GC–MS was performed on the methylated fatty acids using a Finnigan MAT GCQ in full-scan mode with positive chemical ionization. A Restek Rtx-5MS (5% diphenyl–95% dimethyl polysiloxane) column (30 M $\times$ 0.25 mM i.d.) with 0.25-μM film thickness was employed. Helium was used as the carrier gas with 40.0 cm/s flow rate. Fatty acid methyl ester mixture (20 ng) was injected, and the total run time was 34 min. The oven temperature was set at 180 °C, and after 30 min it was programmed to rise to 275 °C at 30 °C/min and then held at 275 °C for 1 min. The structures of *M. ruber* fatty acids were confirmed by comparison of retention times and fragmentation patterns to those of the standard bacterial fatty acid methyl esters (Supelco, Bellefonte, PA).

1′-β-Glucopyranosyl-3,4,3′,4′-tetradehydro-1′,2′-dihydro-β,ψ-caroten-2-one (1): amorphous solid; UV/vis (EtOH) λ_{max} 450 (sh), 478, 506 (sh) nm; IR (CH_2Cl_2) ν_{max} 1650 cm^{-1} (w); ^1H NMR (CD_3OD, 600 MHz) δ 1.26 (6H, s, H-16′, H-17′), 1.31 (6H, s, H-16, H-17), 1.93 (3H, s, H-18′), 1.98 (6H, s, H-20, H-20′), 2.00 (3H, s, H-19′), 2.01 (3H, s, H-19), 2.07 (3H, s, H-18), 2.42 (2H, d, J = 7.4 Hz, H-2′), 3.15 (1H, dd, J = 7.8, 9.0 Hz, H-2″), 3.25 (1H, m, H-5″), 3.28 (1H, dd, J = 4.5, 9.0 Hz, H-4″), 3.36 (1H, dd, J = 9.0, 9.0 Hz, H-3″), 3.52 (1H, dd, J = 2.4, 11.8 Hz, H-6″a), 3.65 (1H, dd, J = 4.4, 11.8 Hz, H-6″b), 4.52 (1H, d, J = 7.8 Hz, H-1″), 5.88 (1H, dt, J = 7.4, 15.4 Hz, H-3′), 6.11 (1H, d, J = 11.6 Hz, H-6′), 6.17 (1H, d, J = 15.4 Hz, H-4′), 6.19 (1H, d, J = 9.9 Hz, H-3), 6.24 (1H, d, J = 11.3 Hz, H-10), 6.32 (1H, d, J = 11.4 Hz, H-10′), 6.35 (1H, m, H-14), 6.35 (1H, d, J = 14.8 Hz, H-8′), 6.35 (1H, m, J = 14.9 Hz, H-14′), 6.39 (1H, d, J = 15.8 Hz, H-7), 6.40 (1H, d, H-12), 6.50 (1H, d, J = 14.9 Hz, H-12′), 6.58 (1H, d, J = 15.8 Hz, H-8), 6.65 (1H, m, H-15), 6.65 (1H, m, H-15′), 6.65 (1H, dd, J = 11.6, 14.8 Hz, H-7′), 6.71 (1H, dd, H-11), 6.72 (1H, dd, J = 11.4, 14.9 Hz, H-11′), 6.98 (1H, d, J = 9.9 Hz, H-4); high-resolution electrospray MS m/z $[M + H]^+$ 727.4552 (calcd for $C_{46}H_{63}O_7$, 727.4573).

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References and Notes

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