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STRUCTURE ELUCIDATION OF THE ALGAL CAROTENOID (3*S*,5*R*,6*R*,3′*S*,5′*R*,6′*S*)-13′-*CIS*-7′,8′-DIHYDRONEOXANTHIN-20′-AL 3′-β-LACTOSIDE (P457). PART 2, NMR STUDIES[1]

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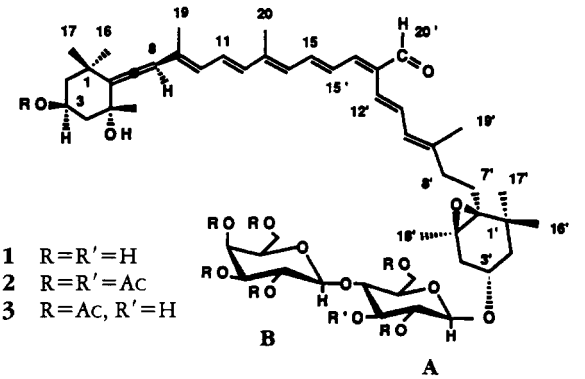
ABSTRACT.—Extensive nmr studies on the algal carotenoid P457 [**1**], its octaacetate **2**, and a heptaacetate **3** resulted in the elucidation of its structure including the C-13′-cis configuration of the cross-conjugated C-20′-al chromophore, the relative stereochemistry of the allenic end group, the presence of an uncommon C-7′,C-8′-single bond, the C-5′,C-6′-epoxide, and of a β-lactoside attached to C-3′ of the carotenoid. P457 [**1**] is one of the most structurally complex carotenoids known, and its ^{1}H- and ^{13}C-nmr data have been fully interpreted.

The carotenoid P457 is a minor carotenoid disaccharide encountered in several dinoflagellates (2,3). Improved methodology has permitted the reisolation and structure elucidation of P457 [**1**].

The mass cultivation of dinoflagellates, the isolation and purification of **1**, and the chemical derivatization and synthesis of relevant model compounds have been presented previously (4). Some preliminary results have also been reported in symposium presentations (5,6). In the present work, nmr data for **1** and two acetylated derivatives [**2** and **3**], essential for structural assignment, are presented.

Structural features that required establishment by extensive nmr studies comprised confirmation of the cross-conjugated C-20′-carotenal chromophore, including its C-13′-cis configuration, and of the novel C-7′,C-8′-dihydro end group. The saturated C-7′,C-8′-structural element exhibited a previously unknown influence on the chemical shifts of this presumed C-5′,C-6′-epoxy end group carrying a diglycoside, tentatively located at C-3′. Identification of the diglycoside, known to consist of glucose and galactose (3) was also required. Moreover, the relative stereochemistry of both the allenic and epoxy end groups required confirmation.



[1]Part 62 in the series Algal Carotenoids. For Part 61, see E.S. Egeland, *et al.* (1).

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RESULTS AND DISCUSSION

By an improved procedure, ca. 1.2 mg of **1** were isolated from pure cultures of *Amphidinium carterae* (4) and purified by hplc. The pure compound was submitted to extensive nmr analysis providing data compatible with structure **1**. The octa-acetate **2** was obtained as the major product upon acetylation with Ac_2O in pyridine. A minor product unexpectedly turned out to be the heptaacetate **3**, demonstrating a stronger resistance of the C-3-hydroxy group of the glucose moiety of the sugar unit towards acetylation. Particularly the heptaacetate **3** proved useful for the identification of the diglycoside moiety by nmr.

No molecular ion could be obtained for **1** by ms. Fabms of **2** and **3**, however, confirmed the molecular formulas assigned.

Of the different nmr techniques applied, 1D TOCSY was best suited for assignment of overlapping protons in the carbohydrate methine region, as well as for the identification of the signals of the methylene protons of both end groups and at the C-7′,C-8′ positions in the carotenoid aglycone that were partly hidden by other strong signals. In addition, 2D ROESY experiments provided valuable information concerning stereochemical aspects.

Treated below in consecutive order are the following nmr assignments: identification of the diglycoside moiety, its attachment to the carotenoid aglycone, the location and configuration of the polyenal chromophore, the methylene protons and substitution pattern of the two cyclic end groups, the relative configuration of the allenic end group, the saturated C-7′,C-8′ structural element, and finally the remaining assignments.

In the heptaacetate **3**, the signals of the two anomeric axial protons were well separated from each other as well as from those of other protons (in contrast to the free glycoside **1** in CD_3OD and the octa-acetate **2** in $CDCl_3$) and served as a good starting point for a series of 1D TOCSY experiments. Some experimental results were presented previously (6). The two carbohydrate units, subsequently assigned to glucose and galactose, are referred to as rings A and B.

A complete sub-spectrum of the protons of ring A in the heptaacetate **3**, was obtained (6) as described in the Experimental. The position of the non-acetylated hydroxy group was located at A-3 on the basis of the high-field shift of H-3a in comparison with the corresponding nmr spectrum of the octaacetate **2**, as well as from

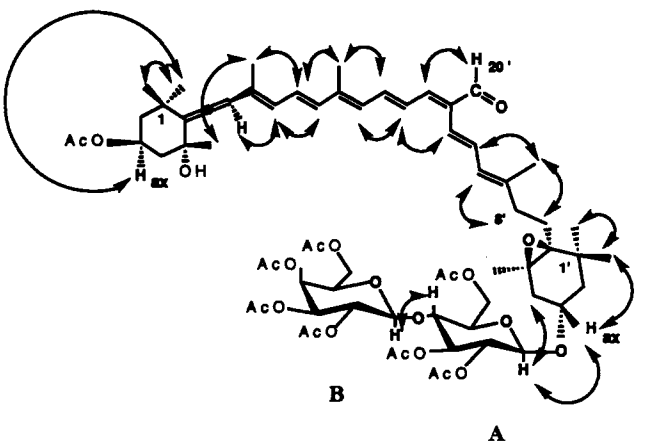

FIGURE 1. Through-space connectivities of **2** measured by 2D ROESY.

the small coupling between OH-3a and H-3a (J=1.7 Hz). The OH signal and the spin splitting disappeared upon addition of D_2O.

Analogous 1D TOCSY experiments were performed starting with the anomeric proton H-1b of the heptaacetate **3**. In this case the transfer of magnetization was interrupted at proton H-4b. The coupling constant $J_{4,5}$ was obviously too small (<1 Hz) for further transfer of the magnetization. The remaining protons H-5b and H-6a,H-6b were localized from cross-peaks of a 2D ROESY nmr spectrum and also in the one-bond ^{1}H-^{13}C COSY spectrum. Additional information on the sequence of the coupled protons in ring B of heptaacetate **3** was also provided by ^{1}H-^{1}H 2D COSY nmr spectroscopy.

In the octaacetate **2**, the signals of the anomeric protons were partly overlapping and hence a sequence of 1D TOCSY experiments was performed starting with other signals that were sufficiently separated such as H-4a, H-5a, H-4b, and H-5b, providing the complete sub-spectra of both rings A and B.

In **1**, the heptaacetate **3** and octaacetate **2**, the vicinal coupling constants between the protons of ring A were between 7.5 and 11 Hz, thus proving their axial orientation and hence the presence of a β-glucose. In agreement with these large coupling constants was the observation that magnetization was readily relayed from H-1a to both protons H-6a. In contrast, magnetization transfer from H-1b was interrupted in ring B at proton H-4b that was clearly equatorially oriented with a small $J_{3,4}$=ca. 3.5 Hz and vanishing coupling constant ($J_{4,5}$<1 Hz).

These results supported the identification of ring B as β-galactose. The presence of glucose and galactose moieties was consistent with the hydrolysis results (4).

From the 2D ROESY nmr spectra of **2** and **3**, an A-4/B-1 linkage of the two carbohydrate rings was demonstrated by cross-peaks between protons H-1b and H-4a (6).

The attachment of the β-lactoside at C-3′ of the carotenoid aglycone was corroborated by the presence of cross-peaks in the 2D ROESY spectra of **2** and **3** between protons H-1a and H-4′eq, as well as H-1a and H-3′ax (overlapped by H-3a in **3** and H-4a in **2**). Attachment of the diglycoside at C-3′ of the carotenoid also followed clearly from a 1D TOCSY spectrum starting from the partly hidden signal of H-3′ in **2**. The expected sub-spectrum of the methylene protons at C-2′ and C-4′ was obtained, typical for C-3-substituted C-5,C-6-epoxy end groups with coupling constants of the expected magnitude, but, as expected, with different chemical shifts compared to related end groups with regular substitution at C-6′ (7). Starting from the signal of H-1′ax in a further 1D TOCSY experiment with **2** using a mixing time of only 10 msec, the signal of H-3′ was clearly identified (line width of ca. 23 Hz) together with that of H-2′eq and, very weakly, of H-4′eq and H-4′ax. In the octa-acetate **2** the attachment of the diglycoside at C-3′ was also proven unambiguously by a cross-peak in the multiple-bond ^{1}H-^{13}C COSY spectrum between H-1a and C-3′ (6).

2D ROESY nmr experiments with **2** and **3** provided further information on the geometry of the polyene chain and the orientation of the aldehyde proton. Relevant through-space contacts, derived from the 2D ROESY spectrum of **2** are depicted in Figure 1 as arrows between linked protons. All expected through-space interactions between protons on the same side of the polyene chain were observed, thus defining the geometry of the polyene chain. Intense cross-peaks between the aldehyde proton H-20′ and H-14′, and between H-15′ and H-12′, confirmed the cis-configuration of $\Delta^{13'}$ and the orientation of the aldehyde proton. It is interesting to note that H-20′ was weakly coupled ($J_{20',12'}$=2 Hz) only to H-12′ along a typical W-coupling path. In order not to overload Figure 1, all further ROESY contacts observed between protons on the carbohydrate moieties were omitted, e.g., those between the axial protons H-1, H-3, and H-5 in rings A and B.

For other cross-conjugated carotenals such as renierapurpurin-20-al (8) we have demonstrated previously that these compounds prefer the cis geometry of the double bond to which the aldehyde is attached (*E*-configuration). Moreover, it seems to be a general phenomenon for such cross-conjugated carotenals that in the nmr solution an additional minor isomer, presumably with the *trans* configuration, is detectable, as evidenced by a weak additional aldehyde signal at lower field. A corresponding singlet without resolvable splitting was also observed in the ^{1}H-nmr spectra of the compounds studied here near 10.3 ppm with ca. 10% intensity, indicating that the minor isomer is in equilibrium with the major C-13′-cis isomer.

The identification of the partially strongly overlapped proton signals at the end groups of the carotenoid aglycone was also mainly based on 1D TOCSY experiments. The experimental procedure and the results were essentially as described previously for other carotenoids (7,9) (see Experimental for further details). Complete sub-spectra of H-2ax, H-2eq, H-4ax, and H-4eq and of the corresponding spin-system at the primed end group were thus obtained. Pairwise linkage was revealed from slightly different geminal coupling constants and/or from the one-bond ^{1}H-^{13}C 2D COSY nmr spectrum that identified protons attached to the same carbon atom. In the 2D ROESY spectrum the axial methyl groups (H-16 and H-16′) at both end groups showed strong 1,3-diaxial interactions with the protons at H-3 and H-3′.

In agreement with previous nmr studies on peridinin and some related carotenoids (10) the relative stereochemistry of the allenic end group could be unambiguously derived by the ROESY experiment (see Figure 1), because cross-peaks of medium intensity were observed between H-19 and H-18. Moreover, all the chemical shifts of the allenic end group were in agreement with those of suitable reference compounds (7,10). Further cross-peaks in the 2D ROESY spectrum of **2** and **3** between methyl protons and neighboring protons on the same side of each of the two carotenoid end groups also contributed to the reliability of these assignments. Unfortunately, the relative configuration of the epoxy end group could not be clearly settled in these experiments.

The uncommon saturated C-7′,C-8′ structural element and its position were confirmed by ^{13}C and DEPT, ROESY, TOCSY, one-bond and multiple-bond ^{1}H-^{13}C 2D COSY nmr techniques. Through-space contacts were identified between protons H-10′ and H-8′ and between the H-19′ and one of the H-7′ methylene protons. The approximate chemical shifts of the ^{1}H-nmr signals of the methylene protons at C-8′ and C-7′ were additionally accessible from corresponding cross-peaks in the one-bond ^{1}H-^{13}C COSY spectra.

The subsequent assignment of all of the ^{13}C-nmr signals of the octaacetate **2** and heptaacetate **3** in $CDCl_3$ was based essentially on ^{1}H-detected one-bond and multiple-bond ^{1}H,^{13}C 2D COSY. Assignments for **1** (solvent CD_3OD) were based on a complete set of experiments as described for the acetates **2** and **3**.

The complete nmr data for **1**, its octa-acetate **2**, and hepta-acetate **3** are given in Tables 1 and 2. The numbering of the carotenoid skeleton is shown for **1**.

The nmr studies, leading to a complete set of nmr data for P457 [**1**], its octaacetate **2**, and heptaacetate **3**, confirmed the structure deduced for P457 (13′-*cis*-7′,8′-dihydroneoxanthin-20′-al 3′-diglycoside) [**1**] on the basis of other evidence (4).

The diglycoside was identified as a β-lactoside, consistent with the methanolysis results (4). An enantiomeric β-L-glycoside cannot be ruled out from the combined nmr and methanolysis data, but is unknown in nature. Moreover, the relative configuration of the allenic end group was from nmr data shown to be the same as for other naturally occurring allenic carotenoids, including fucoxanthin (11), peridinin (10), and neoxanthin (12,13), in favor of 3*S*,5*R*,6*R* configuration for **1** and consistent with cd data for **1** and peridinin (4).

TABLE 1. ^{1}H-Nmr Data for **1–3** Determined by 400 MHz (**1–3**) and 500 MHz (**2**) 1D TOCSY and ^{1}H,^{1}H 2D COSY.[a]

Proton(s)	Compound		
	1 (CD_3OD)	**2** ($CDCl_3$)	**3** ($CDCl_3$)
2ax	1.29, ~t, $J_{2,2}$~12, $J_{2,3}$~11	1.42, dd, $J_{2,2}$=13.2, $J_{2,3}$=9.9	1.42, dd, $J_{2,2}$=13.0, $J_{2,3}$=11.1
2eq	~1.90, ddd, $J_{2,2}$~12.5, $J_{2,3}$~3.5, $J_{2,4}$~1	~2.00, m	2.01, m
3ax	4.20, m	~5.39, m	~5.39, m
4ax	1.35, dd, $J_{4,4}$~13, $J_{4,5}$~11.5	1.52, t, $J_{4,4}$=$J_{4,5}$~12.0	1.52, dd, $J_{4,4}$=13.0, $J_{4,3}$=11.0
4eq	2.18, ~ddd, $J_{4,4}$=12.5, $J_{4,5}$=5.5, $J_{4,2}$~1	2.29, m	2.29, m
8	6.072, s	6.066, s	6.067, s
10	6.17, d, $J_{10,11}$=11.5	6.15, d, $J_{10,11}$=11.5	6.15, d, $J_{10,11}$=11.7
11	6.81, ddd, $J_{11,12}$=15, $J_{11,10}$=11.5	6.70, dd, $J_{11,12}$=14.9, $J_{11,10}$=11.4	6.71, dd, $J_{11,12}$=15.0, $J_{11,10}$=11.4
12	6.42, d, $J_{12,11}$=15	6.37, d, $J_{12,11}$=15.0	6.37, d, $J_{12,11}$=15.1
14	6.41, d, $J_{14,15}$=11.8	6.32, d, $J_{14,15}$=11.9	6.32, d, $J_{14,15}$=11.8
15	7.24, m	7.06, dd, $J_{15,15'}$=13.8, $J_{15,14}$=11.8	7.06, dd, $J_{15,15'}$~14, $J_{15,14}$~11
16	1.336, 3H, s	1.390, 3H, s	1.390, 3H, s
17	1.064, 3H, s	1.077, 3H, s	1.077, 3H, s
18	1.309, 3H, s	1.358, 3H, s	1.358, 3H, s
19	1.852, 3H, s	1.832, 3H, s	1.832, 3H, s
20	2.062, 3H, s	2.043, 3H, s	2.044, 3H, s
2′ax	1.34, dd, $J_{2',2'}$=13.3, $J_{2',3'}$=10.5	1.29, dd, $J_{2',2'}$=12.5 $J_{2',3'}$=10.1	1.30, dd, $J_{2',2'}$=13.1, $J_{2',3'}$=9.3
2′eq	1.66, ddd, $J_{2',2'}$~13, $J_{2',3'}$~3.5, $J_{2',4'}$~1.2	1.62, ddd, $J_{2',2'}$=11.9, $J_{2',3'}$=4.0, $J_{2,4'}$ (*W*)=1.5	1.64, m
3′	3.83, m	~3.74, m	3.72, m
4′ax	1.78, dd, $J_{4',4'}$=14.2, $J_{4',3'}$=8.3	1.63, dd, $J_{4',4'}$=14.2, $J_{4',3'}$=8.1	1.65, dd, $J_{4',4'}$=15.1, $J_{4',3'}$=8.2
4′eq	2.36, ddd, $J_{4',4'}$=14, $J_{4',3'}$=4.0, $J_{4',2'}$=1.4	2.24, dd, $J_{4,4'}$=14.6, $J_{4',3'}$=5.0	2.24, dd, $J_{4',4'}$=15.0, $J_{4',3'}$=3.1
7′A	1.69, ddd, $J_{7',7'}$=17, $J_{7',8'}$=10 and 7.5	~1.67, m	~1.67, m
7′B	1.95, ddd, $J_{7',7'}$=17, $J_{7',8'}$=11 and 6.5	~1.89, m	~1.90, m
8′	~2.30, 2H, m	~2.25, 2H, m	~2.23, 2H, m
10′	5.98, d, $J_{10',11'}$=11.3	5.94, d, $J_{10',11'}$=11.3	5.95, d, $J_{10',11'}$=10.9
11′	7.58, dd, $J_{11',12'}$=15.3, $J_{11',10'}$=11.1	7.55, dd, $J_{11',12'}$=15.4, $J_{11',10'}$=11.1	7.55, dd, $J_{11',12'}$=15.5, $J_{11',10'}$=11
12′	6.46, dd, $J_{12',11'}$~15, $J_{12',20'}$=1.8	6.39, dd, $J_{12',11'}$=15.3, $J_{12',20'}$=2 Hz	6.39, dd, $J_{12',11}$=15.2, $J_{12',20}$~2
14′	~6.97, m	6.80, d, $J_{14',15'}$=11.9	6.80, d, $J_{14',15'}$=11.7
15′	~6.97, m	6.86, dd, $J_{15,15'}$~13.6, $J_{15',14'}$~11.8	6.87, dd, $J_{15,15'}$=13.7, $J_{15',14'}$=11.6
16′	1.064, 3H, s	1.054, 3H, s	1.052, 3H, s
17′	1.226, 3H, s	1.156, 3H, s	1.159, 3H, s
18′	1.384, 3H, s	1.349, 3H, s	1.353, 3H, s
19′	1.848, 3H, s	1.855, 3H, s	1.854, 3H, s
20′	9.49, d, $J_{20',12'}$=2.1	9.535, d, $J_{13',20}$=2.0	9.535, d, $J_{13',12'}$=2.0
	10.300, s, 0.06 H, all-trans	10.307, s, ~0.1 H, all-trans	10.306, s, ~0.1 H, all-trans
A-1a	4.37, d, $J_{1,2}$~7.3	4.50, d, $J_{1,2}$=8.1	4.43, d, $J_{1,2}$=8.1
A-2a	3.19, ~dd, $J_{1,2}$=8.1, $J_{2,3}$=8.5	4.84, dd, $J_{2,3}$=9.6, $J_{2,1}$=8.1	4.83, dd, $J_{2,3}$=9.6, $J_{2,1}$=8.1
A-3a	3.51, dd, $J_{3,4}$~$J_{3,2}$=10	5.18, t, $J_{3,2}$=$J_{3,2}$=9.3	3.72, dd, $J_{3,2}$=$J_{3,4}$=9.6, $J_{3\text{-OH}}$=1.7
A-4a	3.57, t, $J_{3,4}$=$J_{2,3}$=9	3.74, dd, $J_{4,5}$=9.5, $J_{4,3}$=9.0	3.49, dd, $J_{4,5}$=9.5, $J_{4,3}$=8.0
A-5a	3.40, ddd, $J_{4,5}$=9.5, $J_{5,6a}$~$J_{5,6b}$=3	3.59, m	~3.56, m
A-6a,b	3.86, m, 2H	~4.09, m	~4.00, m
A-6a,b		4.46,dd, $J_{6,6}$=11.2, $J_{5,6}$=1.8	4.30, dd, $J_{6,6}$=11.2, $J_{6,5}$=1.6
B-1b	4.37, d, J~7.3	4.48, d, $J_{1,2}$=8.0	4.54, d, $J_{1,2}$=8.0
B-2b	3.54, dd, $J_{2,3}$=8.8, $J_{2,1}$=7.5	5.12, dd, $J_{2,3}$=10.5, $J_{2,1}$=7.9	5.25, dd, $J_{2,3}$=10.5, $J_{2,1}$=8.0
B-3b	3.48, dd, $J_{3,2}$=9.1, $J_{3,4}$=3.1	4.96, dd, $J_{3,2}$=10.4, $J_{3,4}$=3.5	5.00, dd, $J_{2,3}$=10.5, $J_{3,4}$=3.2
B-4b	3.81, d, $J_{4,3}$=3.3	5.35, d, $J_{4,3}$=3.3	5.39, dd, $J_{3,4}$=3.5, $J_{4,5}$<1
B-5b	~3.58, m	3.87, dd, $J_{5,6}$=7.1, $J_{5,6}$=6.5	4.03, m
B-6a,b	3.69, dd, $J_{6,6}$=11.5, $J_{6,5}$=4.5	~4.08, m	4.11, dd, $J_{6,6}$=11.5, $J_{6,5}$=7.5
B-6b,b	3.78, dd, $J_{6,6}$=11.5, $J_{6,5}$=5.5	~4.13, m	4.15, dd, $J_{6,6}$=12.0, $J_{6,5}$=5.1
OH			4.25, d, J_{OH-3}=1.7
Ac		1.968, 3H, s; 2.043, 6H, s; 2.052, 3H, s; 2.063, 3H, s, 2.111, 3H, s; 2.154, 3H, s	1.985, 3H, s; 2.044, 3H, s; 2.060, 3H, s; 2.091, 3H, s; 2.097, 3H, s; 2.100, 3H, s; 2.163, 3H, s

[a]Coupling constants (J) are given in Hz.

TABLE 2. ^{13}C-Nmr Signals (δ) for **1–3** (100 MHz), Determined by DEPT-135, One-bond, and Multiple Bond ^{1}H-detected ^{1}H-^{13}C 2D COSY.

Carbon	Compound			Carbon	Compound		
	1 CD_3OD	**2** $CDCl_3$	**3** $CDCl_3$		**1** CD_3OD	**2** $CDCl_3$	**3** $CDCl_3$
1	36.72[a]	35.85	35.84	A 1	102.76	99.62	99.93
2	49.50	45.46	45.46	2	74.82	71.86	72.58
3	64.72	67.97	68.00	3	72.61	72.91	73.45
4	49.00	45.27	45.27	4	80.65	76.58	82.99
5	73.17	72.71	72.74	5	76.41	72.65	71.72
6	117.30	117.70	117.75	6	61.95	62.26	62.86
7	204.14	202.40	202.72	B 1	105.19	101.16	102.10
8	103.83	103.37	103.37	2	76.52	69.15	68.74
9	135.52[b]	134.10	134.14	3	74.86	71.04	70.91
10	129.28	128.35	128.42	4	70.36	66.66	66.85
11	129.00	127.60	127.64	5	77.13	70.74	71.54
12	137.65	136.59	136.66	6	62.55	60.87	61.76
13	143.74	142.00	142.04	$CH_3C{=}O$		20.57	20.48
14	132.68	131.38	131.46			20.63×3	20.52
15	140.41	138.31	138.36			20.79	20.66
16	29.56	29.22	29.22			20.87	20.67
17	32.94	32.10	32.10			20.92	20.92×2
18	31.39	31.31	31.32			21.45	21.46
19	14.34	14.12	14.12	$CH_3{-}C{=}O$			
20	13.10	13.14	13.14	A-2[c]		169.14	
1′	36.78[a]	35.58	35.59	A-3[c]		169.55	
2′	46.74	45.05	45.19	Other		169.84	169.55
3′	72.85	72.91	72.88			170.12	169.68
4′	39.32	38.42	38.55			170.20	170.04
5′	66.94	64.72	64.86			170.40×3	170.11
6′	70.67	68.95	69.01				170.48×2
7′	30.51	29.35	29.41				170.63
8′	37.96	36.81	36.82				
9′	142.97	142.39	142.49				
10′	127.78	126.34	126.38				
11′	132.60	131.84	131.91				
12′	121.77	120.62	120.67				
13′	135.54[b]	134.37	134.42				
14′	150.14	147.51	147.56				
15′	128.73	127.49	127.57				
16′	26.08	25.70	25.70				
17′	29.34	28.60	28.65				
18′	21.48	21.23	21.25				
19′	17.15	17.34	17.35				
20′	196.03	193.96	194.02				

[a]May be interchanged.
[b]May be interchanged.
[c]Site of attachment.

The relative configuration of the epoxy end group could not be unequivocally assigned by nmr techniques. However, the 3′*S*,5′*R*,6′*S* configuration is preferred from biosynthetic analogy with common carotenoid epoxides with C-7′,C-8′ double bonds (14,15). Moreover, syntheses of relevant model epoxides also supported anti- configuration between the 3′-lactosyl and the C-5′,C-6′-epoxy group in **1** (4).

With its $C_{40}+C_{12}$ carotenoid skeleton, five different oxygen functionalities in the carotenoid aglycone, special structural features including 13-cis configuration, one allenic chiral axis, and five (aglycone) and ten (lactoside) chiral centers, **1** is one of the most structurally complex naturally occurring carotenoids, cf. Straub (14) and Kull and Pfander (15).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The nmr spectra were recorded at 25° in $CDCl_3$ (99.96% D) and, in part, in CD_3OD (100%) from euriso-top (CEA) with TMS as internal standard on a Bruker AM-400 (400 MHz ^{1}H and 100.6 MHz ^{13}C) or Bruker AMX-500 spectrometer. Due to limited sample quantities, excessive acquisition times (up to 90 h) were necessary, in part, to obtain good ^{13}C-nmr spectra. The 500 MHz instrument was used to acquire ^{1}H-nmr spectra of the acetates **2** and **3** in order to resolve overlapped parts. The 1D TOCSY nmr experiments were performed in the 'reverse' mode using the decoupler as the source of ^{1}H-nmr excitation. The DANTE pulse train consisted of 650 up to 2400 short pulses of 2 μsec duration, separated by a delay of 70 μsec at a power level from 18H to 20H (high-power mode). Further details for 1D TOCSY experiments with **3** (and **2**) are given below. In the 2D ROESY experiments a chopped spin-lock of 90° pulses was applied and the delay between the pulses was selected such that a duty cycle of 0.08 was obtained. Typically, the results of 0.4, 0.6, and 0.8 sec mixing times were added. For the one-bond ^{1}H-^{13}C HSQC experiments, pulse sequence *E* (16) was used. The ^{1}H-detected heteronuclear long-range correlation experiment (HMBC) was as described by Bax and Summers (17). In all experiments, standard experimental conditions were used as described in detail previously (6–10).

COMPOUNDS AND DATA.—Quantification of the carotenoids was based on visible spectrometry assuming E (1%, 1 cm) of 2500 at λ max 452 nm in CH_3OH (4). The amounts of the lactoside and its acetates were adjusted according to their mol wts.

P457 [**1**], *(3S,5R,6R,3'S,5'R,6'S)-13-cis-7',8'-dihydroneoxanthin-20'-al 3'β-lactoside.*—Ca. 1.2 mg available; R_f 0.88 (Whatman KC 18F, Me_2CO-CH_3OH-H_2O, 85:10:5); vis (CH_3OH) λ max (424), 452, (480) nm; Ft-ir (KBr) ν max 3350 (OH), 3148–3049 (CH, sp^2), 2957, 2926, 2871, 2855 (CH, sp^3), 1931 (C=C=C), 1654 (cross-conjugated C=O), 1409, 1379 (*gem*-methyl), 1153 (*tert.*) (C-O), 965 (*trans* CH=CH); ms not informative; cd (CH_3OH) λ (Δε) 220 (−6), 229 (−8), 260 (0.4), 278 (−1.2), 310 (−0.2) nm; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

An aliquot of hplc-purified **1** (ca. 0.6 mg) was acetylated with Ac_2O (0.2 ml) in dry pyridine (0.4 ml) under Ar at room temperature for 4 h. The reaction was terminated by pouring the mixture into ice-H_2O containing a small amount of CH_3CH_2OH and diluted acetic acid. The acetylated carotenoids were extracted with ether/hexane (2:1), organic phase was washed with H_2O, and the solvent evaporated. Two products (**2** and **3**) were separated by tlc (two plates, Si gel, EtOAc-hexane, 2:1; R_f 0.45 and R_f 0.35, respectively), desorbed with 5% CH_3OH in Et_2O and the solvent evaporated.

P457 octaacetate [**2**].—R_t 23.5 min, Spherisorb S5-W, 250×4 mm, eluent *n*-hexane-10% EtOAc-2.5% EtOH, 40 bar/1.5 ml/min, detection 455 nm, available ca. 0.4 mg hplc pure; uv vis λ max (CH_3OH) 452 nm; fabms *m/z* 1276 (M^+); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

P457 heptaacetate [**3**].—R_t 27.9 min, above hplc system, available ca. 0.2 mg hplc pure; vis λ max (CH_3OH) 452; fabms *m/z* 1234 (M^+); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; 1D TOCSY experiments were performed as follows:

By inverting the magnetization of the anomeric proton H-1a by a selective 180° DANTE pulse sequence, followed by an MLEV-17 spin-lock, the magnetization of H-1a was partly relayed to its spin-coupled neighbors depending on the duration of the mixing period. From a difference FID (on-resonance minus off-resonance excitation) a sub-spectrum of the irradiated proton and its spin-coupled neighbors was obtained that showed the same digital resolution as the normal 1D nmr spectrum, thus providing accurate nmr data. Several 1D TOCSY nmr spectra with increasing duration of the mixing time (10, 30, 100, 140 msec in four different experiments) were acquired. The magnetization of H-1a was then increasingly relayed to all subsequent protons. At the longest mixing time, the complete sub-spectrum of the protons of ring A was obtained (6). From the appearance of the different proton signals in subsequent experiments, additional information was deduced on their assignment.

For identification of the proton signals of the end groups of the carotenoid aglycone of **3**, as well as of **2**, selective inversion by a 180° DANTE pulse sequence of the magnetization of H-3 and H-3', followed by an MLEV-17 spin lock of between 20- and 80-msec duration, gave complete sub-spectra of H-2ax, H-2eq, H-4ax, and H-4eq, as well as of H-2'ax, H-2'eq, H-4'ax, and H-4'eq, providing accurate chemical shifts and coupling constants for these protons (Table 1).

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