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Graphical abstract

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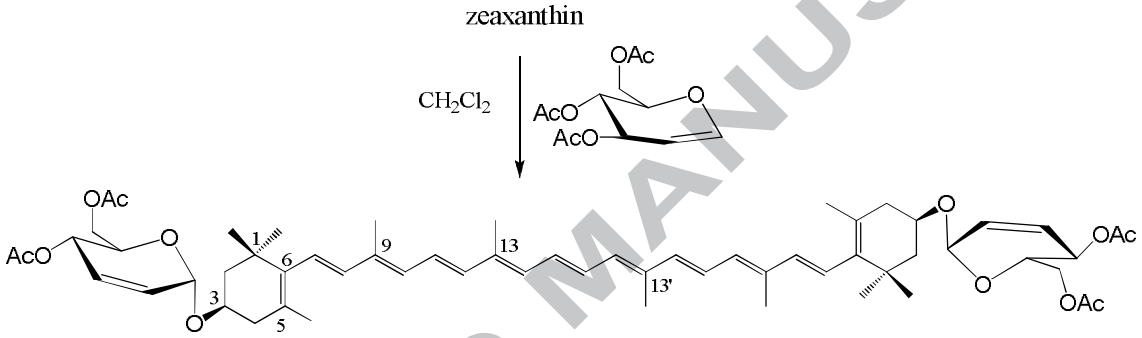

New methods for the synthesis of carotenoid glycosides

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Abstract

We describe our studies on the synthesis of carotenoid glucosides and deoxyglucosides using the acetimidate method and the Ferrier rearrangement, respectively. In both cases the reaction conditions were optimized until the yields were superior to those of previously published glycosylations.

Keywords: carotenoid; glycoside; acetimidate; Ferrier rearrangement.

Thermoxanthins, i.e., C_{40} carotenoid glycosides, have been described as constituents of the cell membrane of certain *Thermus* bacteria, and are believed to be partially responsible for the heat resistance of the *Thermus* species.[1] Carotenoids are also good anti-oxidants, whilst hydrophilic carotenoids are even more so,[2] and therefore thermoxanthins in the cell membrane can play a role in the inhibition of oxidative stress. Since carotenoid glycosides are of limited availability from natural sources,[3] their effect on oxidative stress has not been studied as yet.

Carotenoid glycosides were previously synthesized by direct glycosylation of carotenoid alcohols using the classical Königs-Knorr procedure,[4] and by total synthesis starting from 3-hydroxy-β-ionone.[5] However, these methods gave glycosides with rather low (~3-8%) overall yields. Metabolic engineering using recombinant DNA techniques was also applied for the synthesis of carotenoid glycosides.[6] A new tentative approach for the preparation of thioglycosides as thermoxanthin mimetics was reported in one of our previous papers.[7,8]

Here we describe our results on using modern glycosylation methods for the synthesis of carotenoid glycosides starting from some accessible hydroxy-carotenoids (Figure 1).

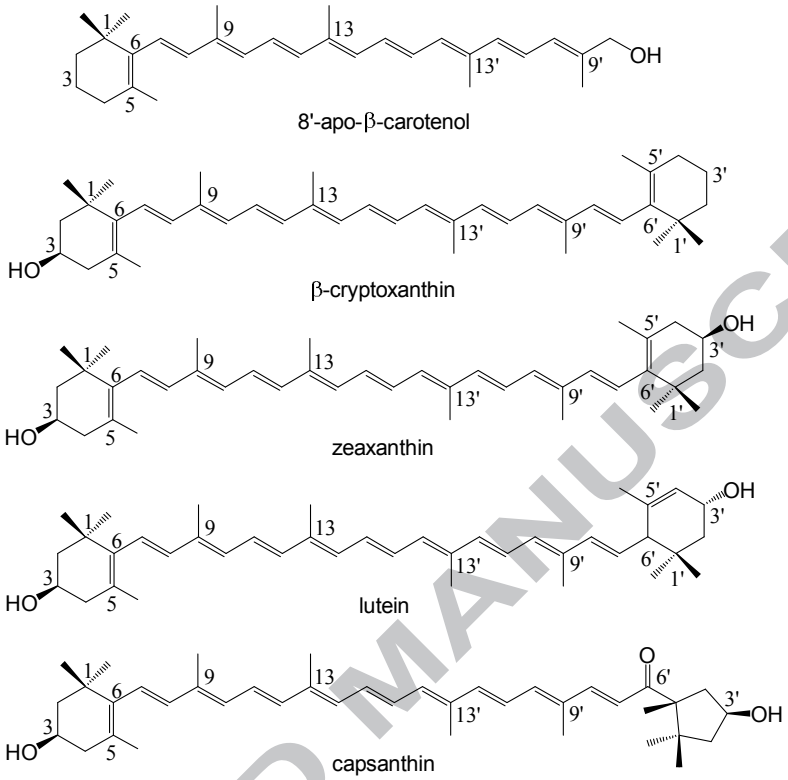
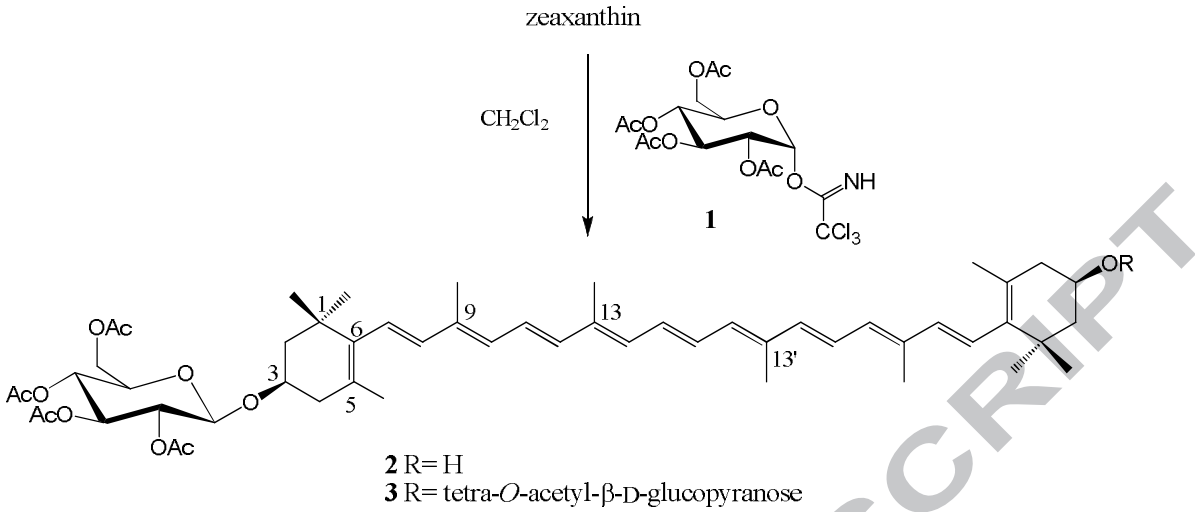


Figure. 1. Hydroxy-carotenoids used in the glycosylation reactions

Initially, the Königs-Knorr reaction starting from β-cryptoxanthin and tetra-*O*-acetyl-β-D-glucopyranosyl bromide promoted by silver triflate, was reproduced and gave the target monoglycoside in ~8% yield. However this does not represent improvement over the previous reactions.

In our first attempts to improve the above yield we reacted the symmetrical diol zeaxanthin, with tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate **1** (synthesized in three steps from D-glucose),[9] which is a common, active, generally used glycosylating agent. Different Brønsted and Lewis acids were applied (5-10 mol%) as catalysts in dry dichloromethane. The results showed decomposition in almost all of the cases and the yields were rather low except with *p*-toluenesulfonic acid (*p*-TsOH), where the monoglycoside **2** was obtained in an excellent 77% yield (Scheme 1, Table 1).



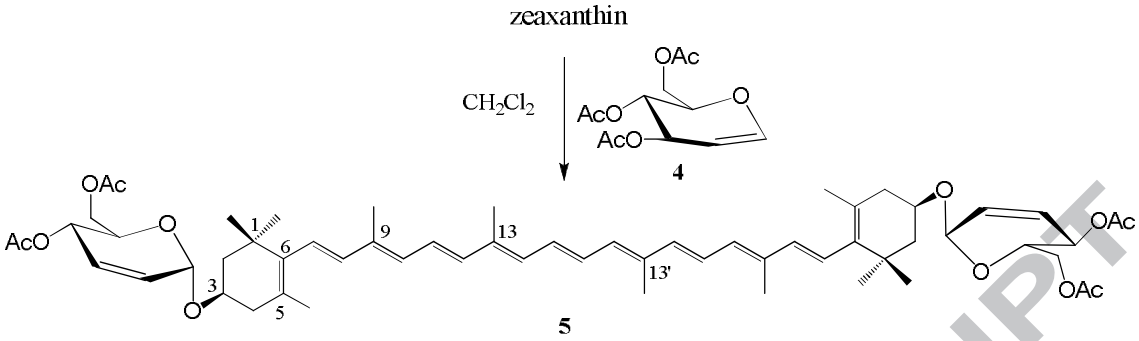
Scheme 1. Mono and diglucosides of zeaxanthin

In all the reactions, at least three equivalents of the sugar were applied and it was added to the reaction mixture in two or three portions. Surprisingly, despite the excess of the sugar donor, only a small amount (6%) of diglucoside **3** formed.

Table 1. Glycosylation experiments of zeaxanthin with tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**1**)

Entry	Catalyst/promoter	Temp.	Reaction time	Products and yields
1	*p*-TsOH	r.t.	overnight	**2** (77%) **3** (6 %)
2	CSA	r.t.	overnight	**2** (10 %)
3	$BF_3 \cdot OEt_2$	0 °C	30 min	**2** (12%)
4	TMSOTf	0 °C	20 min	decomposed
5	CCl_3-CHO	r.t.	24 h	no product observed

The Ferrier rearrangement of tri-*O*-acetyl-D-glucal (**4**) with zeaxanthin, using boron trifluoride as the catalyst, delivered the deoxydiglycoside **5** exclusively. Other catalysts gave only decomposition products (Scheme 2, Table 2.).



Scheme 2. Ferrier reaction with zeaxanthin

Table 2. Glycosylation experiments of zeaxanthin with tri-*O*-acetyl-D-glucal

Entry	Catalyst/promoter	Temp.	Reaction time	Products and yields
1	$ZnCl_2$	r.t.	overnight	decomposed
2	$BF_3{\cdot}OEt_2$	0 °C	30 min	**5** (55%)

The above results with zeaxanthin showed that the best promoter for the glycosylation with the trichloroacetimidate donor was *p*-TsOH, and for the Ferrier reaction was boron trifluoride-diethyl etherate. These conditions were applied for other hydroxy-carotenoids with (8'-apocarotenol and lutein) and without (β-cryptoxanthin and capsanthin) an allylic hydroxy group.

Carotenoids with allylic hydroxy groups are prone to form carbocations especially with Brønsted acids,[10] so some by-products were to be expected. Unfortunately, in these experiments the only reaction that occured was decomposition/dehydration of the carotenoid (Table 3).

Table 3. Glycosylation experiments with other hydroxy-carotenoids

Carotenoid	Sugar	Catalyst/ promoter	Temp.	Reaction time	Yield and products (monoglycoside=MG diglycoside=DG)
β-cryptoxanthin	**1**	*p*-TsOH	r.t.	overnight	**6** MG (8%) + decomposed
capsanthin	**1**	*p*-TsOH	r.t.	overnight	**7** MG (41%) **8** DG (22%)
8'-β-apocarotenol	**1**	*p*-TsOH	r.t.	overnight	decomposed
lutein	**1**	*p*-TsOH	r.t.	overnight	decomposed
β-cryptoxanthin	**4**	$BF_3{\cdot}OEt_2$	0 °C	30 min	**9** MG in trace amounts (<5%)
capsanthin	**4**	$BF_3{\cdot}OEt_2$	0 °C	30 min	**10** DG (32%)

8'-β-apocarotenol	**4**	BF_3 OEt_2	0 °C	20 min	decomposed
lutein	**4**	$BF_3 \cdot OEt_2$	0 °C	30 min	decomposed

Capsanthin gave the monoglycoside (**7**) and the diglycoside (**8**)[11] and also the deoxy diglycoside (**10**) in moderate yields, whereas much to our surprise, in the case of β-cryptoxanthin which bears only one hydroxy group, almost no product formation was observed. It seems that the rate of cation formation and rearrangement of the non-hydroxylated β-end group under the action of acids is comparable with that of the glycosylation.

Based on our results, the dihydroxy carotenoids, zeaxanthin and capsanthin, are the ideal starting materials for acid-promoted glycosylations to give the target glycosides in moderate to good yields in a single step. Whereas natural and synthetic zeaxanthin glucosides are already known, capsanthin glycosides or any κ-carotenoid glycosides have not been described in Nature as yet. Considering the exceptional antioxidant properties of compounds with a κ-end group among the carotenoids,[12] it will be interesting to study how these properties changed in the new derivatives.

Deprotection of the sugars delivers water-soluble carotenoid glycosides. Unfortunately, this has not been achieved yet with acceptable yield. Hence a mild but effective saponification method is still required, especially for the acid-labile deoxyglycosides.

Our results show that modern glycosylation methods can deliver carotenoid glycosides with moderate to good yields, but because of the acidic activation of the donor sugars these methods are restricted to carotenoids bearing non-conjugated hydroxy group(s).

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11. Synthesis of capsanthin glucosides: capsanthin (40 mg, 0.068 mM) was added to trichloroacetimidate (350 mg, 0.71 mM) dissolved in dry CH_2Cl_2 (3 mL) at room temperature under N_2. After complete dissolution, dried *p*-TsOH (8 mg, 0.044 mM) was added. After stirring for 6 h, trichloroacetimidate (175 mg, 0.35 mM) was added again to the solution which was stirred overnight. When TLC showed complete conversion of capsanthin, the mixture was diluted with Et_2O (100 mL) and was washed with sat. $NaHCO_3$ (40 mL) and brine (40 mL). After drying over Na_2SO_4 and evaporation the two main products were separated on a silica gel column. In most cases, subsequent purification on preparative TLC (Merck, Kieselgel 60, eluent *n*-hexane:acetone 7:3) was necessary to obtain the pure red products **7** (25 mg, 41%) and **8** (19 mg, 22%).

Capsanthin tetra-*O*-acetyl-β-D-glucoside (7): UV (λ_{max} nm, EtOH): 476; IR (KBr pellet, cm^{-1}): 3319 m, 1726 vs (νCO, acetate), 1695 vs (conjugated νCO), 1616 s (νC=C), 1246, 1110 vs (νC-O-C). MS (MALDI-TOF) *m/z* = 937.5 (M+Na). ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 0.84 (3H, s, 16'-Me), 1.08 (6H, s, 16,17-Me), 1.21 (3H, s, 17'-Me), 1.37 (3H, s, 18'-Me), 1.74 (3H, s, 18-Me), 1.96-2.2 (30H, m, 19-Me, 19'-Me, 20-Me, 20'-Me, 2-H, 2'-H, acetyl-Me), 2.35 (1H, dd, *J*=17 Hz, 6, 4α-H), 3.01 (1H, dd, *J*=15.5 Hz, 9, 4'α-H), 3.99 (1H, m, 3-H), 4.37 (1H, m, 3'-H), 4.23 (2H, m, 6''-H), 4.95 (1H, d, *J*=10 Hz, 4''-H), 5.20-5.32 (3H, m, 2''-H, 3''-H, 5''-H), 5.74 (1H, d, *J*=6.0 Hz, 1''-H), 6.13 (2H, s, 7-H, 8-H), 6.16-6.75 (11H, m, olefin-Hs), 7.36 (1H, d, *J*=14 Hz, 8'-H). ^{13}C-NMR (125 MHz, $CDCl_3$): 12.7-12.85 (m), 21.5, 21.7, 25.1, 25.6, 28.6, 29.9, 37.0 (1-C), 40.8 (4-C), 43.4 (1'-C), 46.5 (4'-C), 49.0 (2-C), 50.6 (2'-C), 58.5 (5'-C), 63.2, 66.7 (3-C), 69.4, 71.9, 73.2, 73.8, 74.3, 96.9 (1''-C), 120.8, 125.9, 126.2, 129.8, 131.3,

131.6, 132.4, 133.6, 135.1, 135.7, 137.1, 137.3, 137.4, 138.5, 140.7, 142.0, 146.9, 169-170 (C=O, acetyl), 206.8 (6'-C). Analysis calcd. for $C_{54}H_{74}O_{12}$: C 70.87, H 8.15, found C 70.73, H 8.23.

Capsanthin di(tetra-*O*-acetyl-β-D-glucoside) (8): UV (λ_{max} nm, EtOH): 475; IR (KRS-5 window, cm^{-1}): 1738, 1731vs (νCO, acetate), 1674 s (conjugated νCO), 1599, 1581 s (νC=C), 1248, 1124 vs (νC-O-C). MS (MALDI-TOF) *m/z*=1267.4 (M+Na). ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 0.84 (3H, s, 16'-Me), 1.1 (6H, s, 16,17-Me), 1.21 (3H, s, 17'-Me), 1.37 (3H, s, 18'-Me), 1.74 (3H, s, 18-Me), 1.96-2.2 (42H, m, 19-Me, 19'-Me, 20-Me, 20'-Me, 2-H, 2'-H, acetyl-Me), 2.35 (1H, m, 4α-H), 2.95 (1H, m, 4'α-H), 4.04 (1H, m, 3-H), 4.38 (1H, m, 3'-H), 4.20 (4H, m, 6''-H), 4.95 (2H, m, 4''-H), 5.20-5.45 (6H, m, 2''-H, 3''-H, 5''-H), 5.72 (1H, d, *J*=5.3 Hz, 1''-H), 5.75 (1H, d, *J*=5.5 Hz, 1''-H), 6.15 (2H, s, 7-H, 8-H), 6.16-6.75 (11H, m, olefin-Hs), 7.35 (1H, d, *J*=13.5, 8'-H). ^{13}C-NMR (125 MHz, $CDCl_3$): 12.7-12.9 (m), 21.5, 21.6, 25.2, 25.7, 28.6, 29.9, 37.0 (1-C), 40.5 (4-C), 43.4 (1'-C), 46.5 (4'-C), 49.4 (2-C), 50.7 (2'-C), 58.5 (5'-C), 63.3, 67.5 (3-C), 69.7, 71.9, 73.3, 73.8, 74.3, 96.9 (d,1''-C), 120.9, 125.8, 126.1, 129.8, 131.3, 131.6, 132.5, 133.6, 135.1, 135.7, 137.1, 137.2, 137.4, 138.5, 140.8, 142.0, 146.8, 169-170 (C=O, acetyl), 206.7 (6'-C). Analysis calcd. for $C_{68}H_{92}O_{21}$: C 65.58, H 7.45, found C 65.40, H 7.41.

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