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Experiments on the synthesis of carotenoid glycosides

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ABSTRACT

Isozeaxanthin was treated with trifluoroacetic acid and tetra-*O*-benzoyl-1-thio-β-D-glucose to afford β,β-carotene-4,4′-bisthioglucoside isomers in good yields. The deprotected compounds are mimetics of naturally occurring thermoxanthins and can show favourable effects against oxidation stress.

Carotenoids are found in Nature mainly as esters of fatty acids, however, they rarely occur as carotenoid glycosides. Among the pigments of certain heat resistant *Thermus* bacteria, various carotenoid glucosides, the so-called thermoxanthins,[1] were found. Common properties of these thermoxanthins are that the carbohydrate moiety is attached to the 3 and/or 3′ positions of the carotenoid via a glycosidic bond and some of the hydroxy groups of the sugar are esterified with long chain carboxylic acids. In appropriate conformation, the length of the thermoxanthins is equal to the width of the phospholipid bilayer, and due to their amphiphilic structure, they are incorporated into the cell membrane and modify its properties. This particular behaviour of thermoxanthins is believed to be partially responsible for the heat resistance of the *Thermus* species.[1] Carotenoids are also good antioxidants, 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, their effect on oxidative stress has not been studied as yet. This Letter presents a new tentative approach for the preparation of thermoxanthin mimetics.

For the chemical synthesis of carotenoid glycosides only two methods have been published: direct glycosylation of carotenoid alcohols using the classical Königs-Knorr procedure,[2] and a total synthesis starting from 3-hydroxy-β-ionone.[3] Both methods give the target compounds with ~3–8% overall yields. Glycosides of astaxanthin were also prepared by a biosynthetic process.[4]

Several attempts were made using modern glycosylation methods but they were found to be inapplicable for the synthesis of carotenoid glycosides (Scheme 1). Starting from β-cryptoxanthin and tetra-*O*-acetyl-β-D-glucopyranosyl bromide, a silver triflate promoted modified Königs-Knorr reaction gave a complex reaction mixture, which contained the target molecule **1** in ~8% yield. Using the tetra-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate donor, β-cryptoxanthin or zeaxanthin proved to be too weak as acceptors in the presence of camphorsulfonic acid, and no glycoside formation was observed. Isozeaxanthin (**2**) is believed to be a more active acceptor bearing hydroxy groups in allylic positions, and so its coupling with the tetra-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate donor was investigated. The reaction proved unsuccessful, as the catalysts employed (chloral, tosic acid and BF_3–etherate) caused rapid dehydration or decomposition of the carotenoid.

Thereafter a classical reaction[5] of carotenoids led us to the successful synthesis of glycosides. Carotenoids are known to form cationic structures in an acidic medium.[6] The reactions of β-carotene with Lewis acids and that of isozeaxanthin with Brønsted acids were examined and the structures of the products obtained were characterised in detail.[7] These carotenoids were established to give blue-coloured cations (charge +2) on acidic treatment and react with simple nucleophiles readily to form substituted derivatives at position 4 and/or 4′.[8]

With appropriate carbohydrate nucleophiles these cations may form carotenoid glycosides, which can be regarded as mimetics of thermoxanthins. Perbenzoylated glucose derivatives were studied as nucleophiles in reactions with cations formed by acidic treatment of carotenoids. The chromatographic behaviour of the obtained glycosides was very similar to that of the free carotenoids, but the presence of benzoyl protecting groups made the products easily identifiable by UV–vis analysis, demonstrating characteristic absorptions at 230 nm.

β-Carotene was treated with boron trifluoride diethyl etherate and the glucose derivatives sequentially. On using tetra-*O*-ben-

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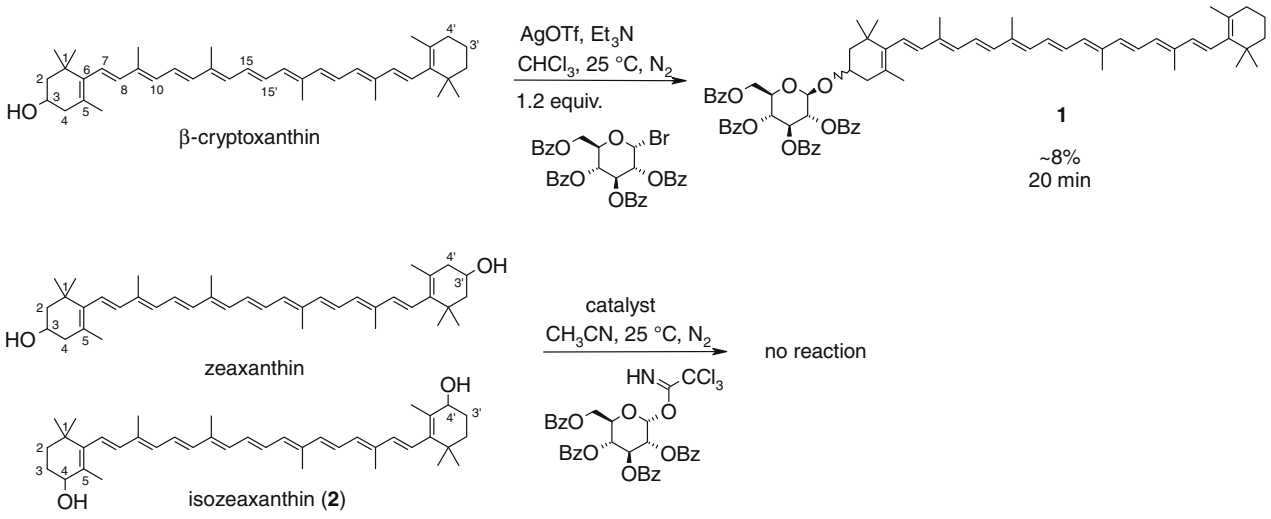


Scheme 1.

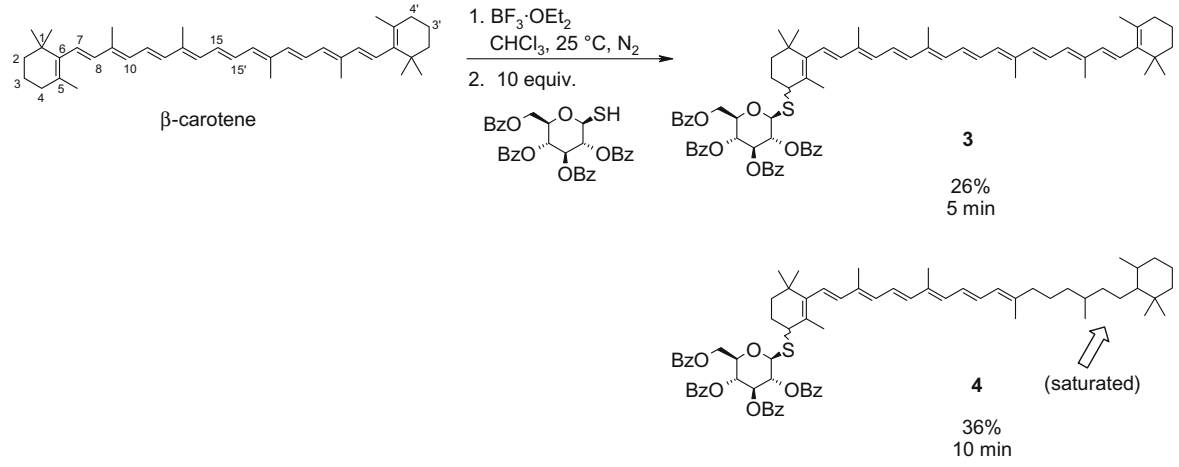
zoyl-α/β-D-glucose no reaction was observed, the glycosidic hydroxy group was not a strong enough nucleophile. The stronger nucleophilic tetra-*O*-benzoyl-1-thio-β-D-glucose gave a successful reaction, depending on the reaction time different products were isolated (Scheme 2). After ~5 min the desired product **3** was detected in 26% of the formed pigment. To improve the yield, a longer reaction time was applied; although the yield of the product increased, saturation of the four double bonds of the polyene system was observed leading to compound **4**, as well (Scheme 3).

β-Carotene reacts with boron trifluoride diethyl etherate via a single electron transfer (SET) mechanism.[7] Under these conditions the 1-thioglucose can behave as a reducing agent, which was proved by the isolation of a bisglucosyl disulfide by-product. Use of a smaller excess of the thiol resulted in lower yields. To avoid the reduction reaction tetra-*O*-benzoyl-α/β-D-glucose was deprotonated with NaH and then treated with the β-carotene dication mixture. Transformation was observed but the products were not identified due to their low quantities in a very complex reaction mixture.

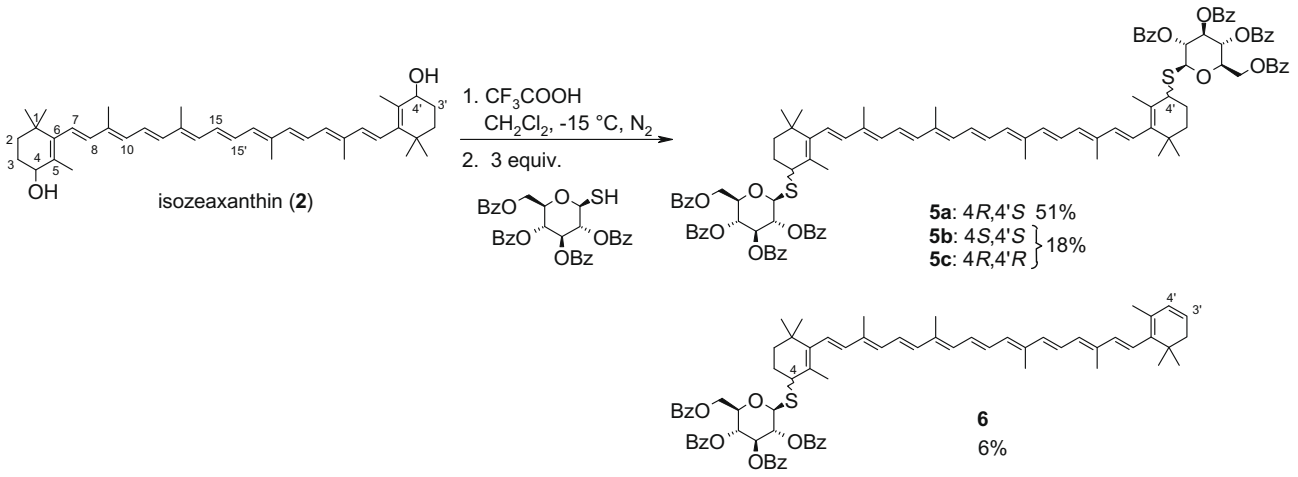
Isozeaxanthin (**2**) reacts with trifluoroacetic acid according to an ionic mechanism to form cationic structures.[9] This reaction medium does not favour reduction of the polyene by thiols, nevertheless, the desired subtitution reactions occur. A dichloromethane solution of isozeaxanthin was treated with trifluoroacetic acid and subsequently with the sugar nucleophile. Tetra-*O*-benzoyl-β-D-glucopyranose again proved to be an inefficient nucleophile and no reaction was observed. However, with tetra-*O*-benzoyl-1-thio-β-D-glucopyranose the expected reaction occurred.[10] HPLC analysis of the crude product showed the formation of three substances, which were isolated and the structure elucidation accomplished by mass spectrometry, NMR and UV–vis spectroscopy.

The main product was identified as benzoyl protected *meso* (4*R*,4′*S*)-β,β-carotene-bisthioglucopyranoside (**5a**) in 51% yield.[11] The diastereomeric pair of perbenzoylated (4*S*,4′*S*)-β,β-carotene-bisthioglucopyranoside (**5b**) and perbenzoylated (4*R*,4′*R*)-β,β-carotene-bisthioglucopyranoside (**5c**) was isolated in 18% yield. These diastereomers were not separated. Compounds **5a–c** formed via a pure ionic mechanism. The reaction intermediate is a planar carbocation,[6–9] and under the reaction conditions there is no possibility for any asymmetric catalytic effect. On the basis of similar subtitution reactions the formation of the 4*R*,4′*S* configured product is the most likely, and the 4*R*,4′*R* and 4*S*,4′*S* configurations form in 25% probability. The third product proved to be 3′,4′-dehydro-β,β-carotene-4-thioglucopyranoside (**6**) in 6% of the pigment. The UV and MS spectra of **6** showed the presence of 12 conjugated double bonds. The chemical shift of the hydrogen atom at position 4 clearly indicates the neighbouring sulfur, thus the newly formed double bond must be at position 3′.

No reduction product was observed. Removal of the benzoyl protecting groups could be achieved using a basic anion exchange



Scheme 2.



Scheme 3.

resin in methanol. During deprotection decomposition of only a small amount of the glycoside was observed. Zemplén conditions failed to remove the protecting groups, and instead resulted in complete decomposition of the products.

The key step in the synthesis of the carotenoid glycosides, that is, coupling of the carotenoid with the sugar moiety was carried out succesfully in reasonable yields. β-Carotene is probably not an ideal candidate as a starting material for the synthesis of glycosides in this way because of the formation of complex reaction mixtures, whereas isozeaxanthin is relatively cheap and easily available starting material which is suitable for the simple one-step synthesis of carotenoid glycosides.

Further study of this method employing other soft-type sugar nucleophiles bearing easily removable protecting groups will be undertaken. The products are sulfur-containing mimetics of naturally occuring thermoxanthins. After deprotection, the antioxidant activity of the carotenoid glycosides on human liver cells will be studied.

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10. A CH_2Cl_2 solution of isozeaxanthin (120 mg) was mixed with TFA (4.8 ml) at −15 °C, under nitrogen in the dark. To the blue solution 3 equiv of tetra-*O*-benzoyl-1-thio-β-D-glucopyranose was added and the mixture was stirred for 40 min. Et_3N was added until the solution became yellow. The mixture was washed with H_2O (50 ml), 5% citric acid solution (50 ml) and brine (50 ml). After drying over $MgSO_4$ the solvent was evaporated and the residue was purified by column chromatography.
11. Characterisation of the main product (*4R,4′S*)-β,β-carotene-bisthio-β-D-glucopyranoside **5a**: λ_{max}: 230, 451, 474 nm. ^{1}H NMR (acetone-d_6, ppm) δ: 8.10 (d, *J* = 7.6 Hz, 4H, Bz-*ortho H*), 7.98 (d, *J* = 7.2 Hz, 4H, Bz-*ortho H*), 7.94 (d, *J* = 8.5 Hz, 4H, Bz-*ortho H*), 7.83 (d, *J* = 8.1 Hz, 4H, Bz-*ortho H*), 7.65–7.34 (m, 24H, Bz-*meta* and *para H*), 6.78–6.72 (m, 5H, polyene chain), 6.47–6.17 (m, 17H, polyene chain), 6.04 (t, *J* = 9.3 Hz, 2H, *H*-3 of glucose), 5.59 (t, *J* = 9.8 Hz, 2H, *H*-4 of glucose), 5.52 (t, *J* = 9.5 Hz, 2H, *H*-2 of glucose), 5.27 (d, *J* = 10.3 Hz, 2H, *H*-1 of glucose), 4.70–4.52 (m, 6H, *H*-5, *H*-6 and *H*-6′ of glucose), 3.94 (pseudo singlet (ps), 1H, *H*-4), 3.62 (ps, 1H, *H*-4′), 2.09, 2.05, 1.99, 1.03, 1.01 (s, 30H, methyl *H* at C-19, C-20, C-18, C-16, C-17 and C-19′, C-20′, C-18′, C-16′, C-17′). MS: 1779.5 $[M+Na]^+$ $C_{108}H_{108}O_{18}S_2$ (MALDI-TOF).