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The first synthesis of PEG–carotenoid conjugates

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ABSTRACT

Carotenoid–PEG esters and diesters were synthesized from several carotenols with polyethyleneglycols of different chain length to enhance the water solubility and bioavailability of these hydrophobic carotenoids. The water solubility of the products was compared and found, as expected, to be proportional with the PEG content of the conjugates.

Carotenoids, being naturally occurring antioxidants and having various biological effects including anti-cancer and cardioprotective, have recently been the focus of food biochemists.[1] Being substantially hydrophobic, and in order to enhance their solubility in water, several succinate, phosphate and amino acid derivatives have been synthesized in the form of di- or tetravalent salts.[2,3] Enhanced water solubility can facilitate their administration as oral antioxidants and is also required for use in some food additives and colorants.

Polyethyleneglycol (PEG) conjugates of a wide range of biomolecules are known (especially peptides).[4,5] These conjugates usually have better pharmacokinetic behavior, and water solubility and, in general, are more efficient in drug targeting. To date, no covalently-bound PEG–carotenoid conjugates have been synthesized, although there are examples of carotenoid–PEG dispersions in the literature which enhance the bioavailability of carotenoids.[6] The advantage of a PEG conjugate over salts is that it changes the osmotic homeostasis much less than ionic compounds. Furthermore, the water solubility of PEG conjugates is independent of pH. If the PEG moiety is connected to the carotenoid through a relatively labile bond, which can be cleaved under physiological conditions, the PEG unit will serve solely as an indifferent, polar carrier for carotenoids (Fig. 1). This property makes such compounds possible nutritional supplements since the esters can be hydrolyzed in the presence of pancreatic secretions to regenerate the parent hydroxy-carotenoids.

We wanted to elaborate the synthesis of the PEG esters of carotenoids to enhance their dispersibility in water, and also to compare the water solubility of products with different carotenoids and PEG chain lengths.

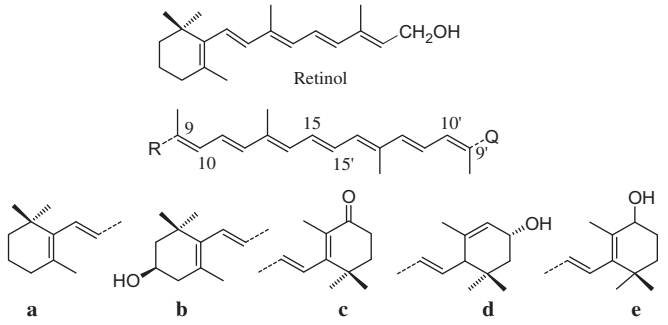

Figure 1. Structures of the carotenoid starting materials: R = **b**, Q = **a**: β-Cryptoxanthin; R = **b**, Q = **d**: Lutein; R = Q = **b**: Zeaxanthin; R = **c**, Q = **e**: 4′-Hydroxyechinenone; R = **a**, Q = CH_2OH: 8′-β-Apocarotenol.

Previously we synthesized a large pool of carotenoid succinates[7] which served as starting materials for carotenoids in reactions with the monofunctional [PEG-550 monomethyl ether (PEG-550-OMe)] and bifunctional [tetraethylene glycol (TEG) and octaethylene glycol (OEG)] derivatives. The carotenoid succinates were coupled with polyethyleneglycols via the Steglich-method[8] using dicyclohexyl carbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) in dichloromethane (Scheme 1).

The reactions with the crystalline carotenoid succinates proceeded smoothly overnight, although in most cases larger amounts of DCC and DMAP were required for the reactions (Schemes 1 and 2). The yields were acceptable especially considering that the products are carotenoid derivatives. Higher polyethyleneglycols are

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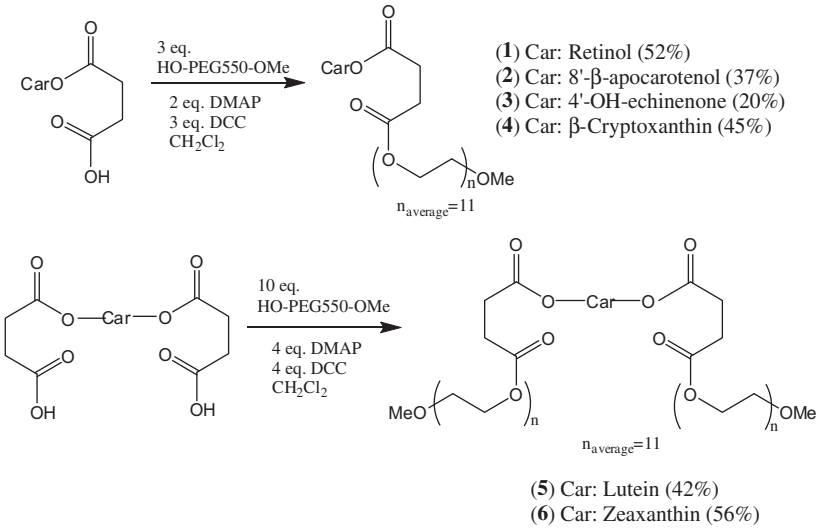
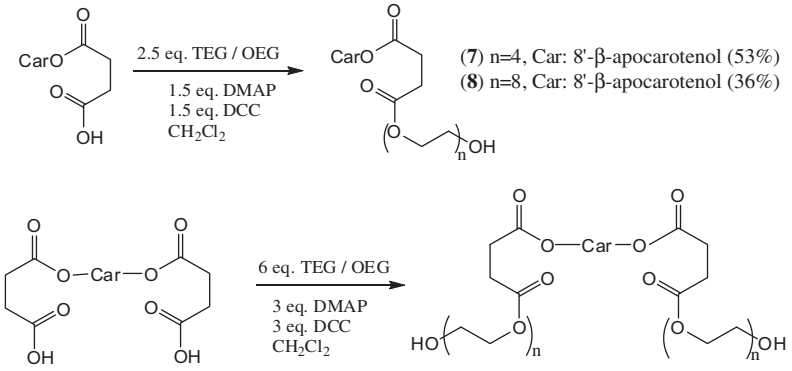


(**1**) Car: Retinol (52%)
(**2**) Car: 8'-β-apocarotenol (37%)
(**3**) Car: 4'-OH-echinenone (20%)
(**4**) Car: β-Cryptoxanthin (45%)

(**5**) Car: Lutein (42%)
(**6**) Car: Zeaxanthin (56%)

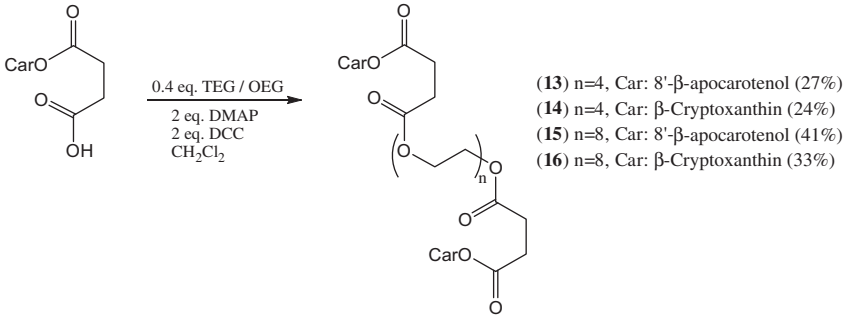
Scheme 1.



(**7**) n=4, Car: 8'-β-apocarotenol (53%)
(**8**) n=8, Car: 8'-β-apocarotenol (36%)

(**9**) n=4, Car: Lutein (39%)
(**10**) n=4, Car: Zeaxanthin (38%)
(**11**) n=8, Car: Lutein (32%)
(**12**) n=8, Car: Zeaxanthin (29%)

Scheme 2.

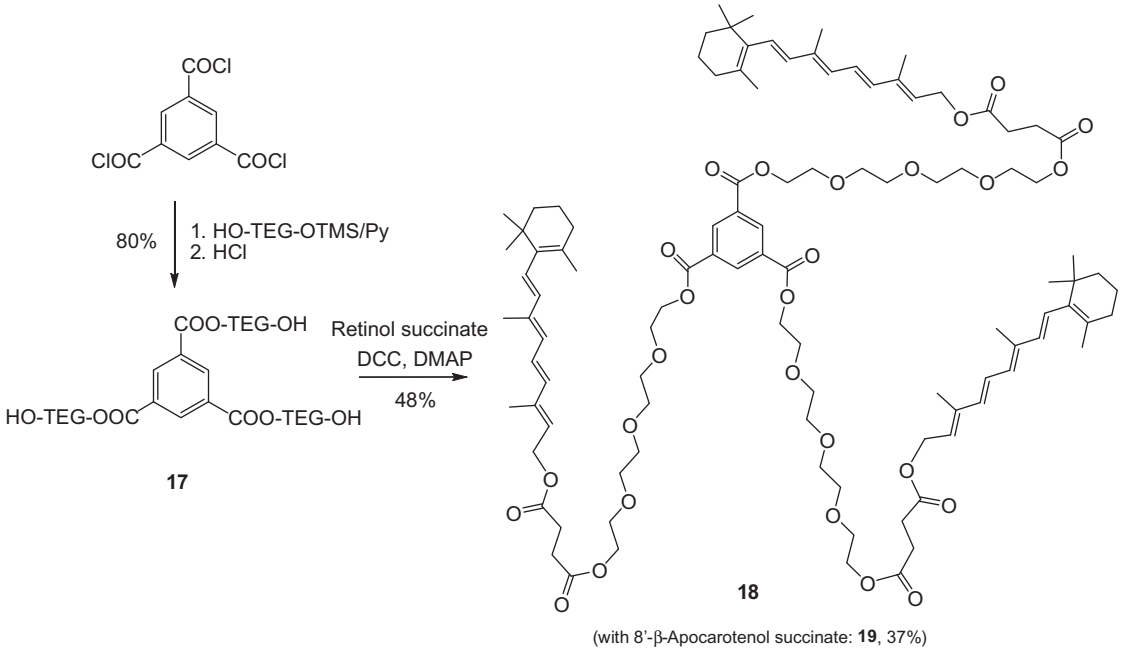


(**13**) n=4, Car: 8'-β-apocarotenol (27%)
(**14**) n=4, Car: β-Cryptoxanthin (24%)
(**15**) n=8, Car: 8'-β-apocarotenol (41%)
(**16**) n=8, Car: β-Cryptoxanthin (33%)

Scheme 3.

available only as a mixture of polymers of different polymerization grade which makes characterization of the products more complicated than those containing TEG or OEG. The readily available monofunctional PEG-550-OMe was chosen for the initial experiments with carotenoid succinates and disuccinates, and the corresponding mono- and diesters were obtained in acceptable yields (Scheme 1).

Using bifunctional PEGs, the reaction was directed to the formation of monoesters with the addition of TEG or OEG in excess (Scheme 2). The yields were again acceptable and the products **7–12** contained free hydroxy functionalities which made further derivatization or coupling possible. On the other hand, when the carotenoid succinates were present in excess the main products obtained were the carotenoid homodimers **13–16** (Scheme 3)



Scheme 4.

Table 1
Comparision of water solubility of some conjugates

Compound	mg/ml (96% EtOH)
2	37.5
4	3.34
6	66
7	2.7
10	2.67
13	0.38

which can be considered as PEG analogs of previously prepared carotenoid dimers.[7]

Previously we synthesized carotenoid triesters with aromatic cores.[9] As conjugates **7–12** bear free hydroxy groups, in theory they could be coupled to aromatic triacids to give PEG analogs of carotenoid trimers (Scheme 4). Direct esterification of **7** and **8** with aromatic triacids or triacyl chlorides did not deliver the trimers. Instead, the TEG (or OEG) moiety was first coupled to a triacyl chloride to give the core molecule **17** which was then esterified with retinol or 8′-β-apocarotenol succinate to give trimers **18** and **19**. All the products were characterized by NMR, UV, HPLC and MALDI-TOF.[10]

To compare water dispersibility, the products were taken up in a minimum amount of 96% ethanol and diluted with water as they were not soluble in water directly. In Table 1 the possible maximum concentrations of the alcoholic solutions are given. These solutions can be diluted with water in any ratio without precipitation of the conjugate. This means that the highest concentration of the aqueous solution (always with some EtOH depending on the extent of dilution) is obtained from **6** which has two long PEG chains. On the other hand, the dimers (such as **13**) with a short TEG/OEG spacer have much lower solubility. These data show that water solubility is proportional to the PEG content of the molecules.

Preliminary test results with liver cell lines showed good antioxidant activity for the new compounds, compared with the corresponding carotenoids, in H_2O_2-induced oxidative stress assays.

Acknowledgments

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10. *General procedure for the synthesis of **9**–**12***: 30 mg of the carotenoid succinate (1 equiv) was added to a solution of TEG/OEG (6 equiv) in 2 ml of dry CH_2Cl_2 under N_2. To the red solution were added DMAP (3 equiv) and DCC (3 equiv) and the mixture stirred overnight. The reaction mixture should be kept in the dark and under N_2. The reaction mixture was poured into Et_2O (100 ml), dried, filtered and evaporated. The polar main product was separated by preparative TLC (Merck, Kieselgel 60, eluent *n*-hexane/acetone, 4:6) to give a red oily product.
*Spectroscopic data for **10***: UV (hexane): 450, 478; MS (MALDI-TOF) m/z = 1121 (calcd for $C_{64}H_{96}O_{16}$ 1120.67). ^{1}H NMR (400 MHz) 1.08–2.1 (m, 38H, methyl Hs, end group Hs), 2.40 (m, 2H, H-4, H-4′), 2.65 (m, 8H, CH_2-succ.), 3.47–3.75 (m, 28H, TEG–CH_2), 4.25 (m, 4H, TEG–CH_2), 5.07 (m, 2H, H-3, H-3′), 6.1–6.7 (m, 14H, olefinic). ^{13}C NMR (125 MHz) δ (ppm) = 12.7, 12.8, 21.4, 24.4–25.5, 28.5, 29.6, 29.7, 30.6, 32.4, 34.0, 36.7, 38.2, 43.9, 49.1, 54.9, 59.0, 61.6, 63.8, 68.8, 69.0, 69.1, 70.3–70.5, 72.5, 124.8–125.5, 130.0, 131.4, 132.5, 134.0–138.6, 171.8, 172.0. Anal. Calcd for $C_{64}H_{96}O_{16}$: C, 68.54; H, 8.63. Found: C, 68.47; H, 8.60.