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Bio-oxidative Cleavage of Carotenoids: Important Route to Physiological Active Plant Constituents

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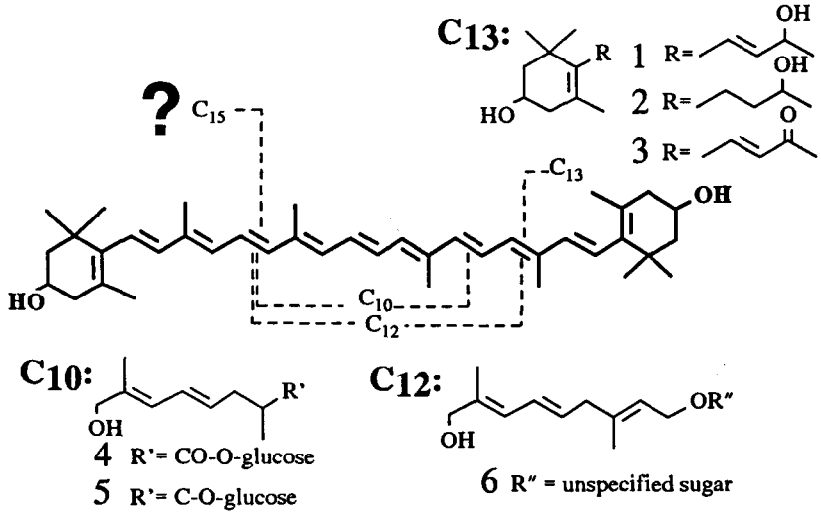
Key Words: Cydonia oblonga; quince; carotenoid metabolism; plant hormones

Abstract: The ß-D-glucosides of (4*R*,1'*E*,3'*E*)-4-(5'-hydroxy-3'-methyl-1',3'-pentadienyl)-3,5,5-trimethyl-2-cyclohexen-1-one **7** as well as of *trans*-abscisic alcohol (ABA-alcohol) **8** have been isolated and characterized in quince fruit through spectral and chemical studies. ABA-alcohol **8** has recently been found to be involved in the biosynthesis of the important plant hormone abscisic acid (ABA) **9**, the latter compound being also present in a still non-specified glycosidically bound form in quince fruit. Based on the finding of these new fifteen carbon (C_{15}) constituents in quince, biodegradation of carotenoids is discussed.

It has previously been shown by our group that quince (*Cydonia oblonga* Mill.) fruit contains a great number of C_{13}-norisoprenoid compounds, such as, e.g., ionone structures 1-3, most of them playing an important role as flavour precursors[1,2]. The additional finding of the irregular terpenoids 4-6[3-5], which are apparently derived from the central part of the carotenoid chain, suggests a hypothetical cleavage of quince carotenoids as schematically outlined below:

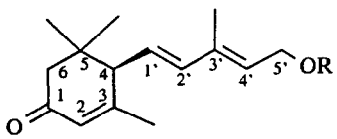


On the basis of the above shown assumed cleavage of quince carotenoids, C_{15}-constituents would be further logical carotenoid metabolites in the fruit. In an effort to isolate these C_{15}-carotenoid end groups, polar extracts of quince juice have been prepared by the following methods:

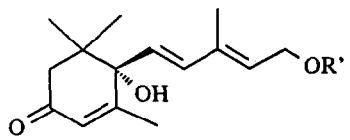
(i) adsorption of glycosidic constituents on Amberlite XAD-2 resin according to the method of Günata *et al.*[6], followed by ethyl acetate elution;

(ii) isolation of non-glycosidic constituents using continuous diethyl ether extraction.

Eluates obtained by method (i) were further fractionated with the aid of rotation locular countercurrent chromatography (RLCC)[7]. After acetylation and flash chromatography[8] a final purification was achieved by preparative HPLC on LiChrospher Si 100 (eluent: Et_2O). In this way, the ß-D-glucopyranoside of ketoalcohol **7** has recently been identified by us as the first C_{15}-structure in quince fruit. More detailed information about the identification of the new natural product **7a** including all spectral data have been published elsewhere[9]. Most recent investigations revealed now the presence of the ß-D-glucopyranoside of ABA-alcohol as further C_{15}-structure in quince[10]. In addition to glucoside **8a** non-conjugated ABA-alcohol **8** was isolated from quince extracts prepared by method (ii)[9]. Whereas glucoside **8a** was identified by us for the first time in nature, aglycone **8** is well-known and has attracted considerable research interest due to its implication in the biosynthesis of the important plant hormone ABA **9**[11,12]. In a recent study it has been shown that alcohol **8** is an intermediate in ABA-biosynthesis in a shunt pathway from ABA-aldehyde involving enzymatic reduction to ABA-alcohol **8** and oxidation of the latter compound to ABA **9** *via* a cytochrome P-450 monooxygenase[11]. The finding of high amounts of glucoside **8a** (approx. 6 mg per kg of fresh fruit) indicates that ABA-alcohol **8** accumulates in form of its ß-D-glucopyranoside in the fruit. However, for quince fruit it is still not known if this accumulation of **8** may generate a precursor pool for subsequent ABA biosynthesis; this possible role of aglycone **8** has previously been suggested by Linforth *et al.*[12]

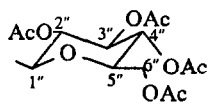


7 R = H

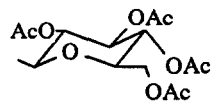


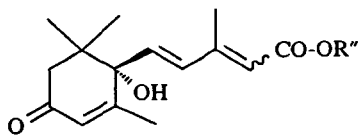
8 R'= H

7a R =



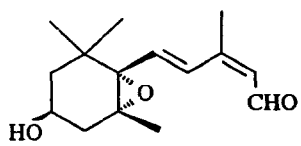
8a R'=





9 R" = H

9a/b R" = CH_3



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The detection of free and glycosidically bound ABA-alcohol **8/8a** prompted us to search also for the presence of the important plant hormone ABA **9** in quince. After enzymatic hydrolysis of separated RLCC fractions followed by methylation of the liberated aglycones with etheral diazomethane[13], two isomers of ABA **9** - tentatively identified as the *cis,trans*- and *trans,trans*-derivatives **9a/b** by comparison with a commercially available reference (*Sigma*) - were identified by HRGC-MS- and HRGC-FTIR-analyses. The final structural elucidation of the conjugating sugar moiety is the subject of active research.

Concerning the biogeneration of the C_{15}-structures under investigation there have been two main approaches to explain their formation from carotenoids. Isoe[14] initially proposed a variety of C_{15}- and C_{13}-degraded carotenoid structures to be derived by photooxidative cleavage of the polyene chain. In recent years, however, biodegradation processes have been favoured, assuming a hypothetical dioxygenase as carotenoid-degrading enzyme[15]. The thoroughly studied enzyme lipoxygenase [EC 1.13.11.12] is such a dioxygenase-type biocatalyst. In the so-called "co-oxidation" process in the presence of certain unsaturated fatty acids this enzyme was found to cleave carotenoids *in vitro* to C_{13}- and C_{15}-fragments[16,17]. Lipoxygenase-catalyzed degradation, e.g., of the ubiquitous epoxy-carotenoid violaxanthin yielded xanthoxin **10** as initial C_{15}-cleavage product[17]. From xanthoxin **10**, which - up to now - could not be detected by us in quince, the enzymatic pathways giving rise to the formation of ABA **9** have been extensively studied and much progress regarding ABA generation from precursor **10** has been made in very recent years[18-22]. Despite these efforts, almost nothing is known about the initial enzymatic cleavage of the carotenoid chain *in vivo*. And since - in addition to lipoxygenase - other carotenoid oxidizing catalytic systems are operative in plant tissues, further investigations are necessary to finally elucidate the important initial step of carotenoid degradation in plants.

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