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CAROTENOID GLYCOSIDES

HANSPETER PFANDER
Institute of Organic Chemistry, University of Berne, Länggassstrasse 7,
CH-3012 Bern, Switzerland

Abstract—Progress in the field of the carotenoid glycosides since 1971 is surveyed. Structures of new natural compounds are discussed. They cover compounds with C_{20}-(crocetin derivatives), C_{30}-(tritperpenoid carotenoids), C_{40}-(zeaxanthin) as well as C_{50}-carotenoids (bacterioruberin, sarcinaxanthin, decaprenoxanthin) as aglycon. Progress in isolating methods, particularly chromatographic methods and the application of countercurrent distribution for the separation of carotenoid glycosides are discussed. Experiments for the partial synthesis of carotenoid glycosides according to the method by Koenigs and Knorr are discussed and the limits of the method shown. Some aspects of the biosynthesis and the function of the carotenoid glycosides, fields in which little is known, are shown.

INTRODUCTION

In the present paper a survey is given of progress in the field of carotenoid glycosides since the publication of O. Isler's book.[1] New structures, progress in the isolation and characterisation, partial synthesis and some biochemical aspects are considered.

Crocin (digentiobiosyl 8,8′-diapocarotene-8,8′-oate) (I) whose structure as digentiobiosyl ester of crocetin was elucidated by Karrer,[2] was the first natural carotenoid glycoside. Although Heilbron[3] as early as 1936, and shortly afterwards Tischer,[4,5] reported the isolation of highly polar carotenoids, it was not until 1967 that Hertzberg and Liaaen-Jensen[6] were able to determine the structures of phlei-xanthophyll (1′-(β-D-glucopyranosyloxy)-3′,4′-didehydro-1′, 2′-dihydro-β,ψ-caroten-2′-ol) and 4-keto-phlei-xanthophyll and show them to be the first real carotenoid glycosides. In the following years the structures of further glycosides were elucidated. Included in the 1971 list of natural carotenoids by Straub[7] are 20 different glycosides, which were mainly isolated from non-photosynthetic bacteria and blue-green algae. The C_{40}-carotenoids form the most important of aglycons. In addition compounds with a C_{50}-skeleton such as corynexanthin[8] (2-[4-(β-D-glucopyranosyloxy)-3-methyl-2-butenyl]-2′-(4-hydroxy-3-methyl-2-butenyl)-ϵ, ϵ-carotene) as well as apo-carotenoids (methyl 1-mannosyloxy-3,4-didehydro-1, 2-dihydro-8′-apo-4-caroten-8′ oate[9]) and diapo-carotenoids (crocin) are also known. The majority of the carotenoid glycosides such as phlei-xanthophyll belong to the group of tertiary glycosides which are very rarely found in nature. In addition, however, secondary and primary glycosides are also known. As sugar residues, D-glucose and L-rhamnose are mainly found.

NEW STRUCTURES

In the course of our investigations on carotenoid glycosides we recently reexamined the pigment composition of saffron (*Croccus sativus*), in particular the water-soluble compounds. Crocin (I), whose presence was reported by Aschoff as long ago as the 19th Century,[10] is the main pigment in saffron (approx. 80%). The mass spectra of the peracetate with a molecular ion at *m/e* 1564 and the characteristic fragments at *m/e* 1217 (M-347), 946 (M-619 + H), 929 (M-635), and the corresponding peaks at *m/e* 635, 619 and 331, demonstrating the loss of mono or disaccharide units respectively, is shown in Fig. 1.

The PMR-spectra is in agreement with the suggested structure, whereby the doublet at 5.95 ppm can be attributed to the anomeric proton of the gentiobiose. The coupling constant of $J \sim 8$ Hz indicates the β-D-configuration. Besides crocin (I) four minor carotenoid glycosides were isolated from the saffron. They are all derivatives of crocetin, having different carbohydrate residues. We isolated the diglucosyl ester (II) (β-D-diglucosyl 8,8′-diapocarotene-8,8′-oate) and the diester in which crocetin is esterified with a molecule each of D-glucose and D-gentiobiose (III) (β-D-gentiobiosyl β-D-glucosyl 8,8′-diapocarotene-8,8′-oate).[11] In addition two monoesters were also isolated, the esters of crocetin with D-glucose (IV) (β-D-glucosyl hydrogen 8,8′-diapocarotene-8,8′-oate) and with D-gentiobiose (V) (β-D-gentiobiosyl hydrogen 8,8′-diapocarotene-8,8′-oate).[12]

The structures were confirmed by PMR-, UV/visible-, IR- and mass spectra (the last only for the peracetates) of the free glycosides and their peracetates. The identification of the carbohydrate residues was achieved by PC, TLC and GC. In addition the peracetates of the digentiobiosyl ester and diglucosyl ester were shown to be identical with the compounds synthesized from α-acetobromoglucose and α-acetobromogentiobiose and the silver salt of crocetin.

Recently Dhingra *et al.*[13] have also reported on the minor glycosides from saffron. They isolated the monoester of crocetin with D-gentiobiose (V) and the diester with D-glucose and D-gentiobiose (III). Their structure determination is mainly based on the identification of several derivatives of the carbohydrate residue and the aglycon, including enzymatic hydrolysis. In addition Dhingra *et al.* also report the isolation of the diester of crocetin with D-glucose and methanol. Since methanol was used as solvent for the extraction and for the chromatography it cannot be excluded that this compound is an artefact. In our experience crocin very easily forms a methyl ester when methanol is used as solvent. The question whether these minor carotenoids isolated from saffron appear naturally in the plant, or whether they are artefacts, is still open. The fact that Kuhn and Karrer isolated only one pigment permits no further conclusions as at that time no chromatographic methods were available and the purification of the compounds was exclusively made by crystallisation whereby minor compounds were lost. We believe that we can exclude the possibility that the compounds described are formed during the isolation in view of the careful chromatographic control of the pigment composition during the whole isolation process.

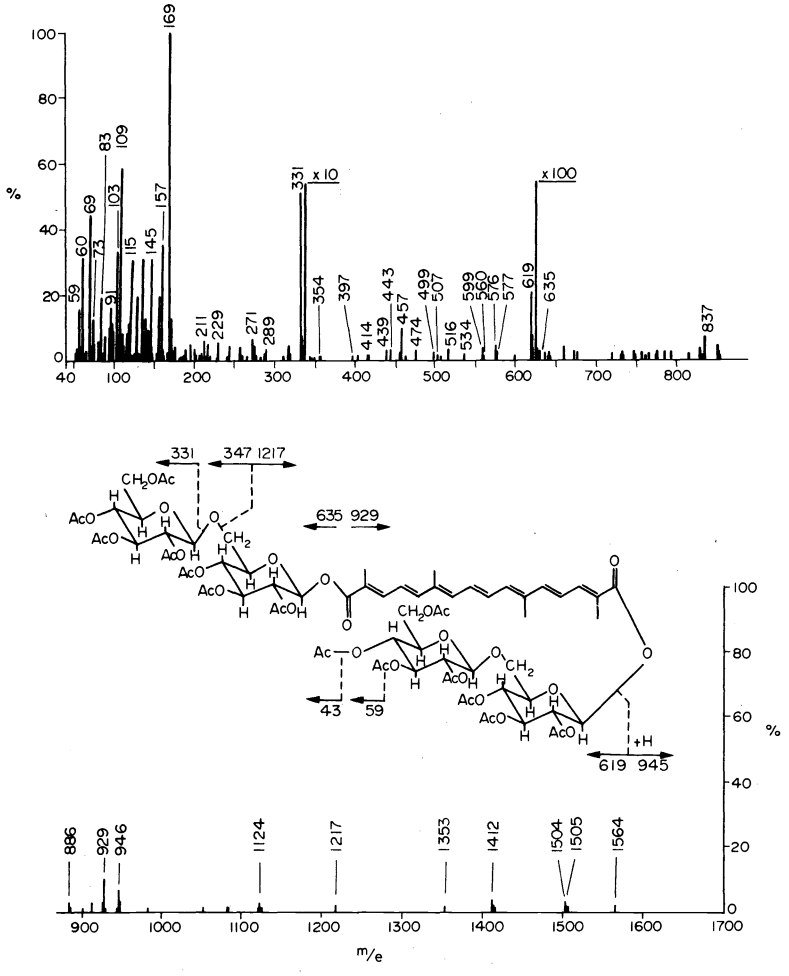


Fig. 1.

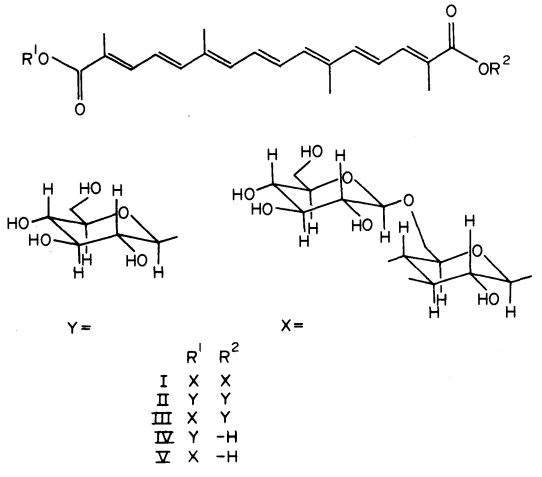


Fig. 2.

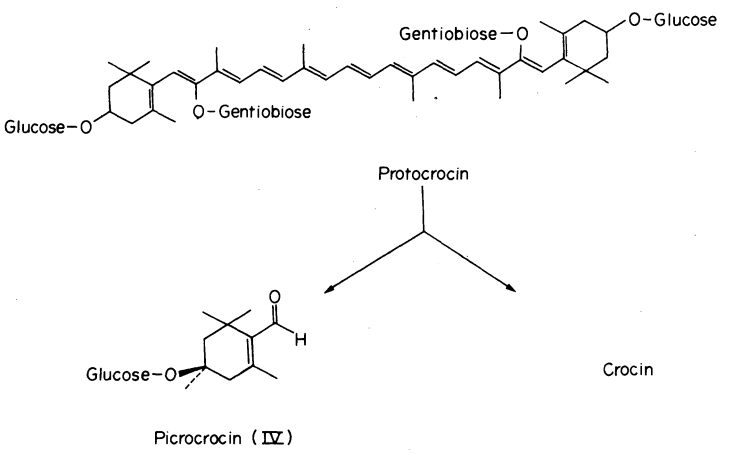
The question, however, remains open whether these minor glycosides are formed between harvest and isolation i.e. in drying.

An indication that besides crocin further crocetin-esters are present in nature is given by the recently carried out investigations on *Croccus albiflorus* Kit.[14] The croccus stigma were immediately extracted following harvest and the carotenoid composition examined. It was found that a series of water-soluble crocetin derivatives appeared whose structure elucidation is at present in progress. The fact that besides crocin, a further glycoside appears in saffron, namely picrocrocin (VI) (3-hydroxy-β-cyclocitral-β-D-glucoside) (Fig. 3) led Kuhn[15] to postulate protocrocin as the common C_{40}-carotenoid precursor of crocin and picrocrocin. The isolation of this hypothetical precursor has to date not been achieved. However, the paper by Buchecker and Eugster[16] who were able to show by comparison with (−)-3-methoxy-β-ionone that picrocrocin has the same chirality at the hydroxylated centre as zeaxanthin at C-3 and C-3′, namely the R-configuration, is a further indication to the existence of a common precursor of crocin and picrocrocin.

During the investigation of the pigment composition of *Streptococcus facium* NNH 564 PH, a non-photosynthetic bacterium, Taylor and Davies[17,18] isolated a novel series of triterpenoid carotenes and xanthophylls among which was a primary glucoside to which the structure of the 4-D-glucopyranosyloxy-4, 4′-diaponeurosporene (VII) (4-D-glucopyranosyloxy-4, 4′-diapo-7,8-dihydro-ψ,ψ-carotene) (Fig. 4) has been assigned.

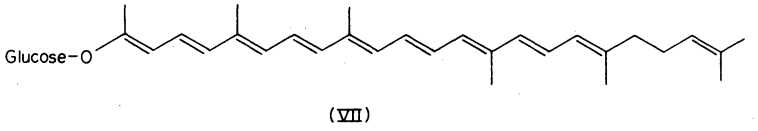
In the C_{40}-series Kleinig and Reichenbach[19,20] isolated further glycosides from myxobacteria. The structure of these new compounds (VIII–XII) are shown in Fig. 5.

The myxobacteria glycosides structurally show certain common characteristics.[21,22] Characteristic of these com-



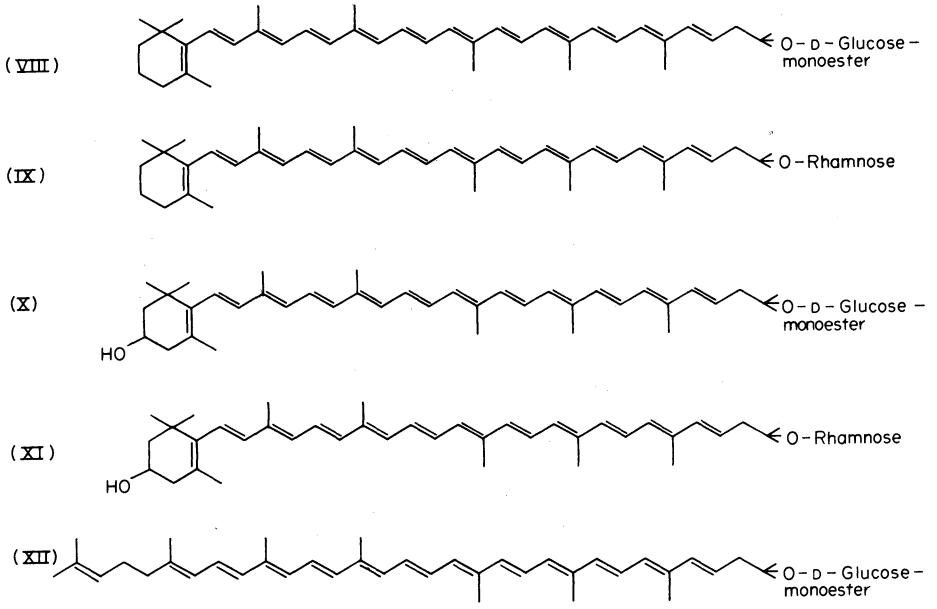
R. Kuhn: 1934
R. Buchecker and C.H. Eugster 1973

Fig. 3.



R.F. Taylor and B.H. Davies 1974

Fig. 4.

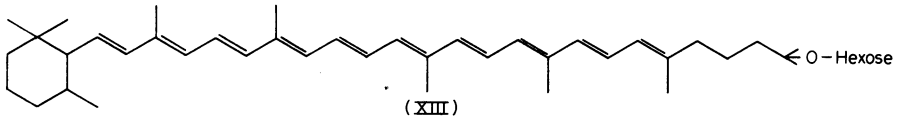


H. Kleinig and H. Reichenbach 1971-73

Fig. 5.

pounds is the formation of the glycosidic bond at the tertiary position C-1′, the double bond between C-3′ and C-4′, as well as the fact that the glucosides normally occur as a monoester of a fatty acid, whereas the rhamnosides are not esterified. Furthermore rhamnosides were isolated from bacteria for the first time, after these had previously been found in blue-green algae. However, the carbohydrate residue is bound to the C-2′ and thus secondary rhamnosides are present.

Myxobactone (1′-glucosyloxy-3′, 4′-didehydro-1′, 2′-dihydro-β, ψ-caroten-4-one) as well as the corresponding compound without the carbonyl group at C-4 (IX) were also isolated by Halfen *et al.*[23] from a gliding organism containing bacteriophyll a and c. In addition a hexoside, presumably a glucoside (XIII), is also described in which, however, and this is interesting to note, the 3′,4′-double bond, characteristic of myxobacteria pigments, is missing (Fig. 6).



(XIII)

L.N. Halfen 1972

Fig. 6.

The first isolation of a secondary non-allylic carotenoid glycoside was successfully achieved by Nybraaten and Liaaen-Jensen,[24] who isolated zeaxanthin monorhamnoside (XIV) ([3R, 3'R]-3'-α-L-rhamnosyloxy-β, β-carotene-3-ol) and zeaxanthin dirhamnoside (XV) ([3R, 3'R]-3, 3'-α-L-dirhamnosyloxy-β, β-carotene) (Fig. 7).

The similarity of the CD spectrum of natural zeaxanthin and of that of the peracetate of the dirhamnoside, permits the conclusion to be drawn that the dirhamnoside has the 3R,3'R-configuration. The α-L-configuration and the 1C conformation of the rhamnose was established by PMR studies.[25]

New C_{50}-carotenoid glycosides have been isolated by Arpin and Liaaen-Jensen. In addition to decaprenoxanthin (XVI) (2,2'-bis(4-hydroxy-3-methyl-2-butenyl)-ε,ε-carotene), and corynexanthin (XVII) which was first isolated by Hodgkiss[26] and which has the structure of decaprenoxanthin monoglucoside,[8] Arpin first isolated the corresponding diglucoside (XVIII) (Fig. 8) from *Arthobacter* Sp.[27]

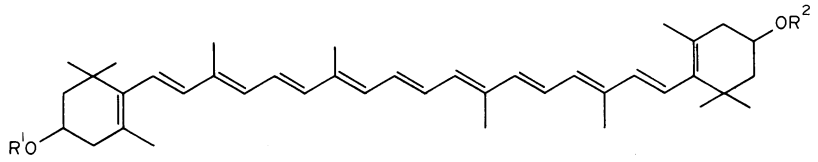
Two further bacterial carotenoid glycosides are the monoglycoside (XX) and diglycoside (XXI) of bacterioruberin (XIX) (2,2'-bis(3-hydroxy-3-methylbutyl)-3,4,3',4'-tetradehydro-1,2,1',2'-tetrahydro-ψ-,ψ carotene-1,1'-diol), the characteristic carotenoid of halophilic bacteria, which were also isolated by Arpin.[28] Besides glucose, mannose was also established as carbohydrate residue. (Fig. 9).

To be formulated in agreement with the revised structure of sarcinaxanthin (XXII) ([2R, 6S, 2'R, 6'S]-2, 2'-bis(4-hydroxy-3-methyl-2-butenyl])-γ, γ-carotene), which shows the γ-end group, is its monoglucoside (XXIII), which was isolated at an earlier stage from *Sarcina lutea*[29,30] and the diglycoside, presumably the diglucoside (XXIV), now found for the first time.[25] (Fig. 10).

Recently Johansen *et al.*[31] reported on the first isolation of a carotenoid glycoside from Dinophycea. The compound P-457, whose structure has not so far been determined, is presumably a hexoside.

ISOLATION AND CHARACTERISATION

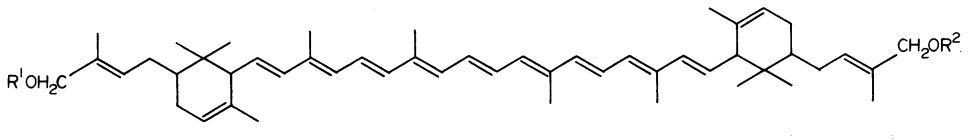
In view of the strong polar character of the carotenoid glycosides, conventional chromatographic methods of carotenoid chemistry such as column chromatography on alumina or silica gel, are not very suitable. The generally poor chromatographic properties, and the fact that in many cases a high lipid content appears, (e.g. 27) make the isolation of carotenoid glycosides extremely difficult so that the separation often has to be effected with the peracetates. During the first isolations distribution between petroleum ether/methanol (80–90%), and subsequent column chromatography on cellulose,[6,9,32] proved to be the most suitable method. As a further adsorbent, magnesium silicate[32] or calcium carbonate[8] were occasionally used. For purposes of comparison and for purity tests, paper chromatography with kieselguhr or aluminium oxide paper[6] proved suitable.



	R^1	R^2
	H	H
XIV	Rhamnose	H
XV	Rhamnose	Rhamnose

G. Nybraaten and S. Liaaen-Jensen 1974

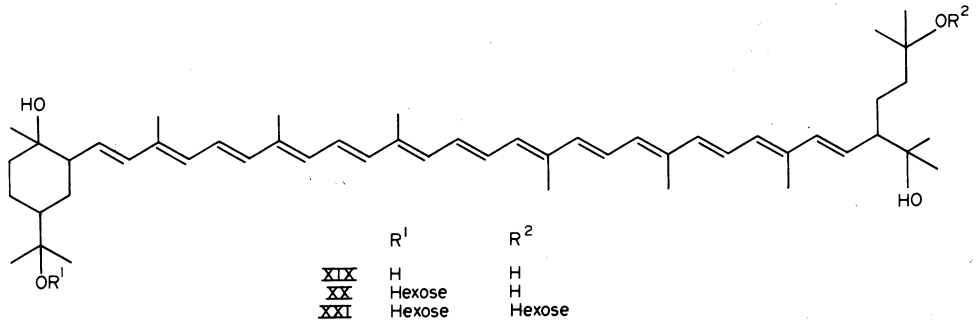
Fig. 7.



	R^1	R^2
XVI	H	H
XVII	Glucose	H
XVIII	Glucose	Glucose

O.B. Weeks and A.G. Andrewes 1970
N. Arpin, S. Liaaen-Jensen and M. Trouilloud 1972

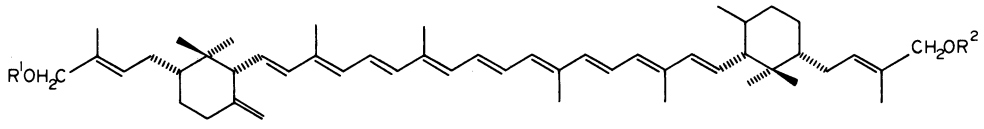
Fig. 8.



	R¹	R²
XIX	H	H
XX	Hexose	H
XXI	Hexose	Hexose

N. Arpin, J.L. Fiasson and S. Liaaen-Jensen 1972

Fig. 9.



	R¹	R²
XXII	H	H
XXIII	Glucose	H
XXIV	Glucose	Glucose

S. Hertzberg and S. Liaaen-Jensen 1975

Fig. 10.

A major advance in the isolation of carotenoid glycosides was achieved by Arpin[27,28] by the use of acetylated polyamide for column chromatography. With the use of increasing content of methanol in benzene as eluent, decaprenoxanthin (XVI),[27] bacterioruberin (XIX)[28] and zeaxanthin[24] could, for instance, be separated from their monoglycosides and diglycosides.

Since, as already mentioned, the peracetates show better chromatographic properties than the glycosides, peracetylation is an important step in isolation. The peracetates can be separated with column chromatography on deactivated alumina or by means of paper chromatography. Kleinig and Reichenbach[33] used magnesium oxide with success for the thin-layer chromatography of the peracetates, while for column chromatography a mixture of magnesium oxide and kieselguhr proved to be most suitable.

At an earlier period we investigated the application of countercurrent distribution to the separation of carotenoid mixtures[34–36] and since then this method has been applied with success as a routine procedure in our laboratories. We have now also applied this method for the separation of carotenoid glycosides. With water as stationary phase and butanol as mobile phase, crocin (I) could be isolated in pure form from the saffron extract and directly crystallised. The minor glycosides were highly concentrated during the separation and a large part of the colourless, accompanying substances separated which greatly facilitated further isolation. Countercurrent distribution could also be used for the separation of zeaxanthin from zeaxanthin monoglucoside (XXV) ([3R, 3′R]-3′-β-D-glucosyloxy-β, β-carotene-3-ol) and zeaxanthin diglucoside (XXVI) ([3R,3′R]-3,3′-β-D-diglucosyloxy-β,β-carotene).[37] As can be seen from Fig. 11 a complete separation could be achieved in a phase pair consisting of 60% acetone, 29% petroleum ether and 11% water in which the compounds to be separated show

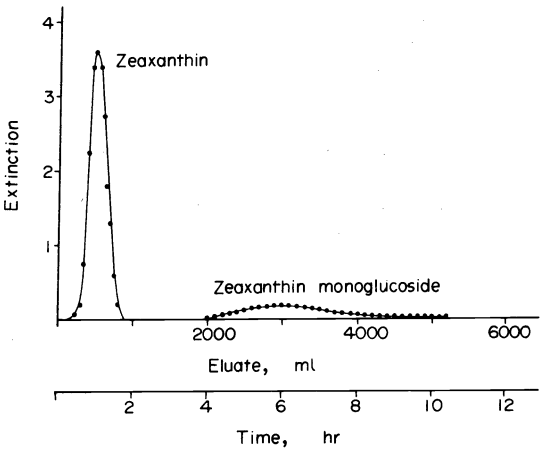


Fig. 11.

distribution coefficients of 3.40 (zeaxanthin), 0.37 (monoglucoside) and 0.017 (diglucoside). While zeaxanthin and zeaxanthin monoglucoside are eluted completely separated in the mobile phase, the zeaxanthin diglucoside can subsequently be isolated from the stationary phase.

Thus, in our experience, countercurrent distribution provides a further method for the isolation and separation of carotenoid glycosides. The separation can be carried out with the exclusion of oxygen and light and no losses occur by adsorption.

For the structural elucidation and characterisation of the carotenoid glycosides and their derivatives mass spectrometry proved to be very useful. The prominent peaks arising from the cleavage of the glycosidic linkage gives first information about the carbohydrate residue.[29] Information about the stereochemistry of the glycosidic bond can be obtained from NMR-spectra.[38,37]

Attempts to define the anomerism of the glucose moiety

by the use of specific glucosidases have up to now been unsuccessful. This was ascribed mainly to the insolubility of the carotenoid glycosides in the reaction mixture. Taylor and Davies have recently[18] shown that the reason for these failures appears to be the specificity of the enzymes.

PARTIAL SYNTHESES

Syntheses or partial syntheses of carotenoid glycosides appear of interest from several aspects. On the one hand, to have them available in the isolation and structural elucidation of new compounds, on the other hand to have available larger quantities which can be applied for the study of the properties of carotenoid glycosides such as their stability or water solubility. With the exception of the partial synthesis of 2′-keto-phlei-xanthophyll tetraacetate from synthetic 1′,2′-dihydro-1′-hydroxy-2′-keto-torulene and α-acetobromoglucose according to the method by Koenigs and Knorr by Hertzberg and Liaaen-Jensen[6] no such experiments have been made.

We have therefore examined various glycosidation reactions on their application in carotenoid chemistry.[39] However, we were only able to observe glycosidation with the method of Koenigs and Knorr. With this method[40] an alcohol or phenol is reacted with a peracetylated glycosyl bromide or chloride in the presence of a heavy-metal salt, normally a silver salt.

We first investigated the reaction of zeaxanthin with α-acetobromoglucose. With a view to possibly obtaining a high yield, the standard conditions for glycosidations of steroid alcohols according to Wulff[41] were applied. The reaction was thus carried out at −14°C in diethyl ether and with the addition of silver carbonate. After a few hours the first products were observed chromatographically, the maximum yield, however, was only achieved after twenty days. Starting with 113 mg zeaxanthin we were able to isolate as major products 15 mg of zeaxanthin monoglucoside pentaacetate (XXVII) and 18 mg of zeaxanthin diglucoside octaacetate (XXVIII). The peracetates were subsequently transferred into the corresponding glycosides (XXV, XXVI) by alkaline hydrolysis.

The NMR-, u.v./visible-, i.r.- and mass spectra are consistent with the postulated structures. In the NMR-spectrum the doublet, at 4.46 ppm with a coupling constant of J ~ 8 Hz indicates the β-D-configuration which is in agreement with the suggested mechanism for this reaction by Wulff.[41] The structure of the minor products could not be fully elucidated. The u.v./visible and mass spectra are identical with those compounds possibly show the α-D-configuration. As a result we investigated further carotenoids and were able to isolate the peracetylated glucosides from lutein (β,ε-carotene-3,3′-diol), 15,15′-didehydro-10′-apo-β-caroten-10′-ol as well as from vitamin A alcohol.[42]. The method by Koenigs and Knorr can thus be applied for the glucosidation of primary and secondary as well as for tertiary hydroxy-carotenoids. In our experience, the applicability is very limited. The generally low yield (maximum approx. 65%), but above all, the very poor reproducibility of the reaction, in spite of careful control of the reaction conditions, make the method for the synthesis of larger quantities appear unsuitable. Moreover it was shown that the course of the reaction is highly dependent on the properties of the silver salt and, in addition, extremely sensitive to impurities. No reaction could be observed with certain carotenoids, as for instance astaxanthin (3,3′-dihydroxy-β,β-carotene-4, 4′-dione), and in individual cases orthoesters are formed as reaction products.[43]

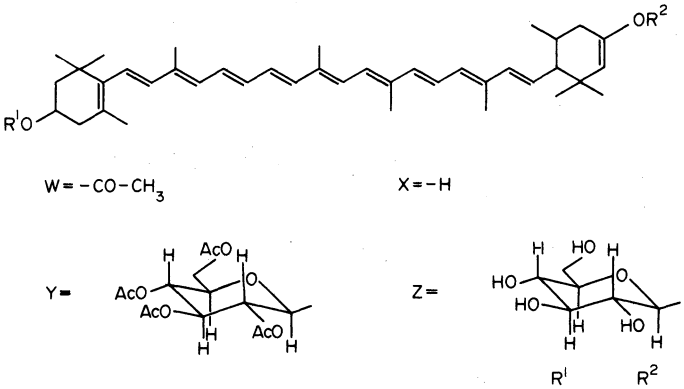
We have investigated the stability and water solubility of the zeaxanthin glucosides in some detail.

The zeaxanthin monoglucoside showed a water solubility of approximately 100 ppm (10 mg/100 ml) at room temperature, while the diglucoside showed one of approximately 800 ppm (80 mg/100 ml). The glucosides proved to be remarkably stable which corresponds to the observation by Nybraaten[24] on the zeaxanthin rhamnosides. Our experiments showed an interesting result insofar as the diglucoside proved to be considerably more stable in water, and more resistent to hydrolysis, than the monoglucoside. Thus the extinction coefficient of a solution of the monoglucoside in water at room temperature and in daylight dropped to 25% of its original value after 14 days; with the diglucoside the drop was only 3%. At pH = 2.90 as well as on pH = 9.0 considerable quantities of zeaxanthin could be observed after 14 days from the monoglucoside, while the diglucoside proved to be stable under these conditions.

BIOCHEMICAL ASPECTS

Although more than 30 different natural carotenoid glycosides are known today, there are at present few results on their biosynthesis or function.[44,45]

Based on the pigment composition in a certain organism, possible pathways for the biosynthesis of



		R^1	R^2
XXVII	Zeaxanthin β-D-monoglucoside pentaacetate	W	Y
XXVIII	Zeaxanthin β-D-diglucoside octaacetate	Y	Y
XXV	Zeaxanthin β-D-monoglucoside	X	Z
XXVI	Zeaxanthin β-D-diglucoside	Z	Z

Fig. 12.

carotenoid glycosides have been suggested. The fact that, besides 4,4′-diapo-neurosporene as the most highly unsaturated carotene, its 4-hydroxy and 4-glucosyloxy-derivative (VII) also appear in *Streptococcus* prompted Taylor and Davies[18] to give a possible biosynthetic pathway. Conditions are similar also for phlei-xanthophyll and 4-keto-phlei-xanthophyll, where the structures of the minor carotenoids also indicate a possible pathway.

Experiments for the elucidation of the biosynthesis of the carotenoid glycosides in *Myxococcus fulvus* were carried out by Kleinig and Reichenbach.[46-48] They investigated the inhibitory effects of nicotine, CPTA (2-(chlorophenylthio)triethylamine hydrochloride), DPA (diphenylamine) and the herbicide San 6706 on the carotenogenesis in this organism which shows myxobacton ester as the major pigment. Based on their experiments with the addition of nicotine and subsequent re-incubation under aerobic and anaerobic conditions they propose the pathway shown in Fig. 13.

The fact that in certain cases 7,8-dihydro-glucoside esters such as 1′-glucosyloxy-3′-, 4′-didehydro-1′,2′,7′,8′-tetrahydro-ψ,ψ, carotene (XXIX)[49] were also found, gives an indication that these compounds are intermediates in the myxobacton ester pathway. The question, however, at present remains fully open as to which is the substrate molecule for the glucosidation.

The biosynthesis of retinol glycosides was described by Gaede *et al.*[50,51] It was found that homogenates of thyroids, liver and intestines catalyse the reaction between sugar nucleotides and retinol:

XDP − sugar + retinol → retinol-glycoside + XDP

where UDP- or GDP-monosaccharides can be applied as sugar nucleotides; so far glucose-, galactose-, mannose and xylose-derivatives have been investigated.

The function of the carotenoid glycosides is at present still completely unknown. Kleinig[52] was able to show that the carotenoid glycoside myxobacton ester is localised as a major pigment in the cell membrane of *Myxococcus fulvus*. Whether the glycoside acts here as a stabiliser of the membrane structure, as a protecter against photodynamic destruction,[53] or carries out other functions, still remains a completely unsolved question.

OUTLOOK

At the time of the isolation of the first carotenoid glycosides, the methods for the elucidation of their structure were not available. The improvement of isolation methods and the development of spectroscopic methods, particularly mass spectrometry, led to the elucidation of the structure of a series of known carotenoid glycosides in the years after 1967 and further compounds were isolated and characterised. With the methods at our disposal today, further natural carotenoid glycosides will doubtlessly be found in the future, either in the reinvestigation of the pigment composition of organisms already examined, or in the investigation of new sources.

Further efforts will doubtless be made in the future in the direction of synthesis. Certainly work will be undertaken on the biosynthesis and the function of carotenoid glycosides, topics which are still largely unexplored. Carotenoid glycosides thus represent a wide-open field for research for the chemist interested in analysis or synthesis, as well as for the biochemist.

Acknowledgements—In conclusion I should like to express my most sincere appreciation to my collaborators who have done the work originating from our laboratory in the field of carotenoid glycosides. They are given in alphabetical order: Dr. Martin Hodler (zeaxanthin glucosides), Miss Yvonne Nussbaumer and Miss Laura Sigg (Glycosidations) and Mr. Fritz Wittwer (crocetin derivatives). I am also most grateful to Dr. G. Englert, Dr. W. Vetter and Mr. W. Meister for running the NMR- and mass spectra, and especially to F. Hoffman-La Roche and Co. Ltd (Basle) for their kind support which made part of our work possible.

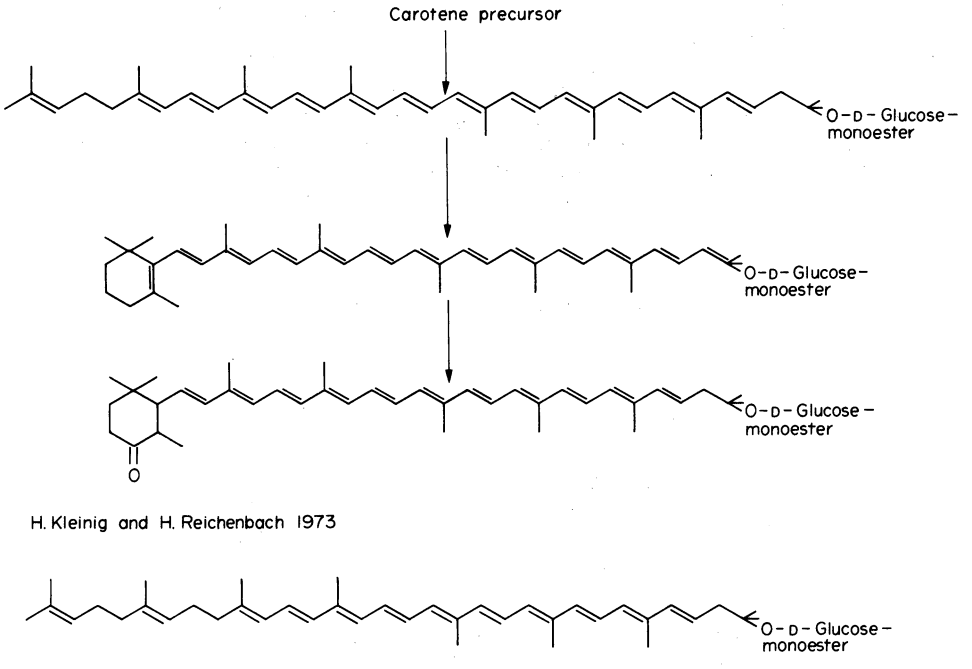


Fig. 13.

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