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CAROTENOIDS OF HIGHER PLANTS—II.*

RUBIXANTHIN AND GAZANIAXANTHIN

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Abstract—Structure I, previously assigned by Kuhn and Grundmann[1] to rubixanthin, is supported by further spectroscopic and chemical data. Except for the melting point and minor deviations in the NMR-spectra and CD-curves, identical data were obtained for gazaniaxanthin. Our observations are in agreement with the recent conclusion by Brown and Weedon[14] that rubixanthin is all-*trans* I and gazaniaxanthin the corresponding 5′,6′-*cis*-isomer. Our comparative NMR-studies provide support for placing the hydroxyl group in the 3-position.

RUBIXANTHIN, first isolated in 1934 from *Rosa rubigenosa* by Kuhn and Grundmann,[1,2] was ascribed structure I, 3-hydroxy-γ-carotene on the basis of the evidence available. I.r. data and its failure to yield allylic oxidation products[3] appeared to support this structure. However, structure I for rubixanthin has not been unequivocally established.

In the present work rubixanthin was found to be present in *R. rubiginosa* exclusively as an ester. The visible spectrum λ_{max} 439, 462 ($\epsilon = 160{,}600$) and 492 nm in acetone and i.r. spectrum (Fig. 1) of rubixanthin corresponded to published data.[1,3] The methyl signals of the NMR spectrum (Table 1) further confirmed that rubixanthin is a hydroxylated γ-carotene, and the molecular formula (M = 552) was confirmed by the mass spectrum. Rubixanthin provided a monoacetate with NMR properties listed in Table 1. From the data presented for γ-carotene, zeaxanthin (II, R = H) and zeaxanthin diacetate (II, R = Ac) it is revealed that small, but significant differences in the chemical shifts of the methyl substituents are observed for unsubstituted, 3-hydroxylated and 3-acetylated β-rings. Hydroxylation causes a slight paramagnetic shift of the *gem.* dimethyl groups as well as of the olefinic methyl group, cf. Barber *et al.*[4] Acetylation results in different shielding of the two *gem.* dimethyl groups, a fact of diagnostic value for the assignment of hydroxyl groups to the β-ring. Finally the olefinic methyl group at C-5 shows a slight paramagnetic shift in the acetate relative to the free alcohol. The NMR data for rubixanthin and its acetate confirm the assignment of the hydroxy group of rubixanthin to the β-ring based on biological vitamin A assay[1] and supported by i.r. data.[3]

The expected doublet at *ca.* 7·70τ ($J = 7$ cps) for the allylic methylene in 4-position (cf. alloxanthin[5] and zeaxanthin Table 1) is, in γ-carotene derivatives, partly masked by the

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* No. 1. *Phytochem.* 7, 839 (1968).

† On leave of absence from Microbiologie et Mycologie, Service de Phytochimie, Université de Lyon.

[1] R. KUHN and C. GRUNDMANN, *Ber.* **67**, 339 (1934).

[2] R. KUHN and C. GRUNDMANN, *Ber.* **67**, 1133 (1934).

[3] S. LIAAEN-JENSEN, E. HEGGE and L. M. JACKMAN, *Acta Chem. Scand.* **18**, 1703 (1964).

[4] M. S. BARBER, J. B. DAVIS, L. M. JACKMAN and B. C. L. WEEDON, *J. Chem. Soc.* 2870 (1960).

[5] A. K. MALLAMS, E. S. WAIGHT and B. C. L. WEEDON, *Chem. Commun.* 301 (1967).

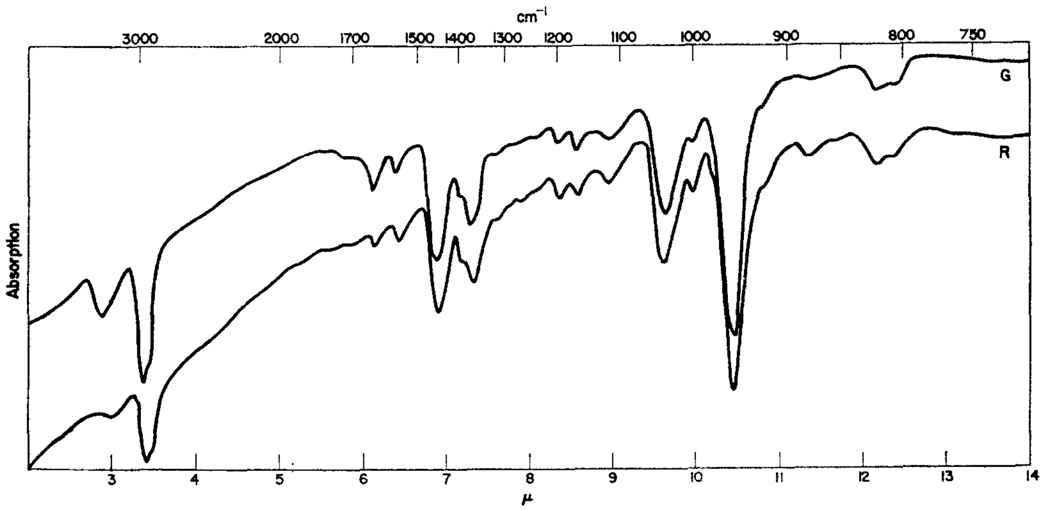
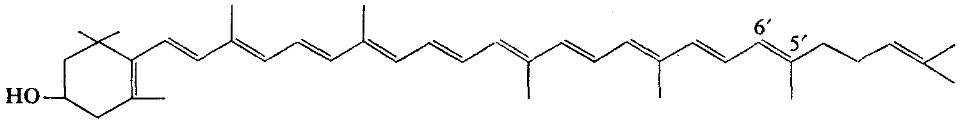
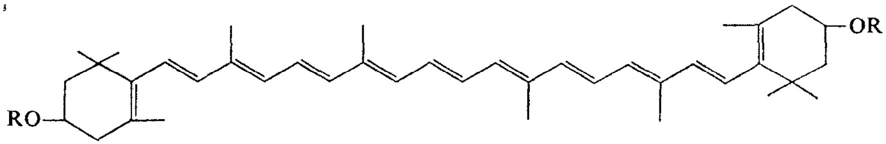


FIG. 1. INFRARED SPECTRA OF RUBIXANTHIN (R) AND GAXANIAXANTHIN (G) IN KBr-PELLET.

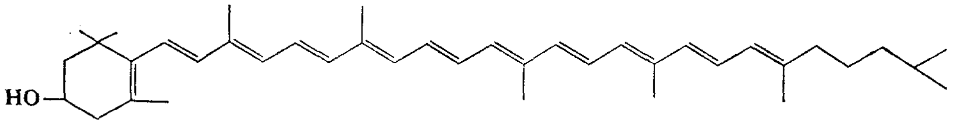
signals of the allylic methylene groups of the aliphatic end-group, but corresponds with that here observed for zeaxanthin. The exact correspondence in chemical shifts of the ring methyl groups in the series γ-carotene–zeaxanthin–zeaxanthin diacetate and γ-carotene–rubixanthin–rubixanthin acetate further suggests that rubixanthin is hydroxylated in the 3- rather than 2-position. A hydroxyl in the 4-position is definitely ruled out from negative results for dehydration[3] and oxidation with *p*-chloranil. The present evidence is therefore taken to support the structure assigned to rubixanthin (I) by Kuhn and Grundmann.[1]



(I) Rubixanthin



(II) R = H Zeaxanthin



(III)

Gazaniaxanthin was first isolated by Schön[6] from flowers of Portugese-grown *Gazania rigens*, where it occurred together with rubixanthin. The two compounds differed in chromatographic adsorptivity (alumina column), crystalline shape and melting point (see Table 2).

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[6] K. SCHÖN, *Biochem. J.* 32, 1566 (1938).

TABLE 1. NMR-SIGNALS OF VARIOUS CAROTENOIDS CONTAINING β-RINGS IN CCL_4

Substance	τ-value (60 Mc/sec)									
	Conj. olefinic	$\genfrac{}{}{0pt}{}{\mathrm{Me}}{\mathrm{Me}}{>}\mathrm{C{=}C}\mathit{H}$—	C*H*OH	O*H*	Allylic CH$_2$	CH$_3$COO	In-chain CH$_3$	CH$_3$ in 5-position β-ring	$\genfrac{}{}{0pt}{}{\mathit{CH}_3}{\mathit{CH}_3}{>}\mathrm{C{=}CH}$—	Non-allylic CH$_2$	*gem.* CH$_3$ β-ring
γ-carotene	3·2–4·2	*ca.* 4·85			7·87 7·92		8·03	8·28	8·31 8·38	*ca.* 8·5	8·93
Zeaxanthin*	3·2–4				7·70 7·80 (7·98)		8·03	8·26		*ca.* 8·6	8·90
Zeaxanthin diacetate	3·2–4				7·64 7·76 (7·95)	(8·0)	8·03	8·24		*ca.* 8·5	8·85 8·90
Rubixanthin	3·2–4·2	*ca.* 4·9	*ca.* 6·2		7·75 7·80 7·86 7·92		8·03	8·26	8·32 8·38	*ca.* 8·6	8·90
Rubixanthin acetate	3·2–4·2	*ca.* 5			7·65 7·77 7·89 7·93	(8·0)	8·03	8·24	8·32 8·38	*ca.* 8·5	8·85 8·90
Gazaniaxanthin	3·2–4·2	*ca.* 4·9	*ca.* 6·1	6·52	7·69 7·80 7·86 7·92		8·03	8·26	8·31 8·38	*ca.* 8·6	8·90
Gazaniaxanthin acetate	3·2–4·2	*ca.* 5			7·65 7·77 7·89 7·93	(8·0)	8·03	8·24	8·31 8·38	*ca.* 8·5	8·85 8·90

* Deuterochloroform.

TABLE 2. CRYSTALLINE SHAPE AND MELTING POINT OF RUBIXANTHIN, GAZANIAXANTHIN AND THEIR ACETATES

Property	Rubixanthin	Gazaniaxanthin	Rubixanthin acetate	Gazaniaxanthin acetate
Crystalline shape from benzene–methanol (*ca.* 1:4)	Leaves and broad needles, Fine needles in star-like clusters*, Kuhn *et al.*[1] Needles; Schön[5] Jagged, elongated plates*,‡	Rectangular leaflets†; Schön[6] Lens-like or plates with rounded edges, occasionally needles in rosettes; Zechmeister *et al.*[7] Elongated plates†,‡	Plates in clusters†,‡	Star-shaped needles; Schön[6] Plates*,‡
Melting point °C	160; Kuhn *et al.*[2] 160; Schön[6] 155–156*,‡	136–137; Schön[6] 133–134; Zechmeister *et al.*[7] 130–138‡, Australian source 131‡, Californian source	138–139*,‡	83–85; Schön[6] 96–97*,‡ 96–97 and 139*,‡,§
	Mixed 131–151‡		Mixed 100–131‡	

* Free of methanol of crystallization.
† Containing 1 mole of methanol of crystallization.
‡ *Present investigation.*
§ Two melting regions.

Schön suggested that gazaniaxanthin might be isomeric with rubixanthin (I) with the hydroxy group in the aliphatic side-chain.

Zechmeister and Schroeder[7] later examined Californian-grown *G. rigens*, which contained only gazaniaxanthin. A general study of the *cis-trans* isomerization behaviour was reported, and on the basis of further analytical data including ozonolytic isopropylidene determination and hydrogenation data structure III was proposed. The inconsistency of the ozonolysis data with the saturated aliphatic end group was pointed out by Karrer and Jucker.[8] More recently Valadon and Mummery[9] has claimed the presence of gazaniaxanthin and absence of rubixanthin in *G. rigens* grown in England.

In the present investigation *G. rigens* grown in Australia was examined and, judged from the melting point, found to contain gazaniaxanthin only. Later, gazaniaxanthin isolated by Zechmeister and Schroeder[7] was put at our disposal. Further investigations on the structure of gazaniaxanthin and a direct comparison with rubixanthin have thus become possible.

As reported by others[6, 7] the shape and position of the visible spectrum of gazaniaxanthin correspond to those of rubixanthin, although some deviation in extinction coefficient is observed ($\epsilon = 142{,}400$ at 462 nm in acetone for gazaniaxanthin). The extinction coefficient measured here for a repeatedly recrystallized sample of gazaniaxanthin is in good agreement with that reported by Zechmeister and Schroeder,[7] since acetone values are generally *ca.* 5 per cent lower than those in hexane. No structural differences are revealed by the i.r. spectra of the two compounds (Fig. 1) or of their acetates. The methyl signals of the NMR spectrum of gazaniaxanthin and its acetate (Table 1) further demonstrated that gazaniaxanthin contains an isopropylidene end-group (τ 8·32, 8·39; 6H) and must be a γ-carotene hydroxylated in the β-ring (cf. arguments for the position of the hydroxyl group of rubixanthin above). In $CDCl_3$ solution, a singlet at τ 6·51 (1H) was attributed to the hydroxyl proton by heavy-water technique. No differences are seen in the CCl_4 spectra of rubixanthin acetate and gazaniaxanthin acetate, whereas certain small deviations exist in the allylic methylene region (τ 7·6–8) and the non-allylic methylene region (around τ 8·5) for the two alcohols. However, the NMR spectra of neither compound justifies the assignment of the hydroxyl to 2-position. Also in the case of gazaniaxanthin a hydroxyl group in the 4-position can be ruled out on the basis of a negative response towards oxidation with *p*-chloranil.

The mass spectra of rubixanthin and rubixanthin acetate, reported and discussed elsewhere,[10] were in agreement with structure I for rubixanthin. The corresponding spectra obtained for gazaniaxanthin and its acetate were identical.

Trans-rubixanthin and "*trans*"-gazaniaxanthin could, in our experience, not be separated in any chromatographic system tested (Table 3). We can offer no satisfactory explanation for the successful separation reported by Schön on alumina, since separation on aluminium oxide-paper employing the circular technique, which in our experience provides superior separation, failed. Nor were any significant differences observed for the chromatographic or spectral properties of the iodine catalysed stereomutation mixtures (Table 4). Likewise no separation of the stereoisomeric sets obtained on iodine catalysed isomerization of the *trans* acetates were observed (Table 4).

The difference in crystalline shape reported by various authors[1, 6, 7] argues against the identity of rubixanthin and gazaniaxanthin (Table 2). In the present investigation no major

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[7] L. Zechmeister and W. A. Schroeder, *J. Am. Chem. Soc.* **65**, 1535 (1943).

[8] P. Karrer and E. Jucker, *Carotinoide*, p. 323, Birkhäuser, Basel (1948).

[9] L. R. G. Valadon and R. S. Mummery, *Phytochem.* **6**, 983 (1967).

[10] C. R. Enzell, G. W. Francis and S. Liaaen-Jensen, *Acta Chem. Scand.*, in press.

TABLE 3. CHROMATOGRAPHIC PROPERTIES OF *trans*-RUBIXANTHIN, GAZANIAXANTHIN AND THEIR ACETATES

Carotenoid	Required eluant from neutral alumina activity grade 2	R_f value						
		Schleicher & Schüll No. 287*			Schleicher & Schüll No. 288†		Schleicher & Schüll No. 996‡	Whatman SG 81§
		1%§	2%	5%	2%	10%	2%	5%
Rubixanthin	20–25% acetone in petrol. ether		0·39	0·64		0·36		0·49
Gazaniaxanthin			0·39	0·64		0·36		0·49
Rubixanthin acetate	30% ether in petrol. ether	0·63			0·52		0·76	
Gazaniaxanthin acetate		0·63			0·52		0·76	

* Kieselguhr paper; † aluminium oxide paper; ‡ calcium carbonate paper; § kieselgel paper.

difference in crystal shape was observed under the microscope when the free alcohols and their acetates were crystallized from benzene–methanol under similar conditions (Table 2). Presence of methanol of crystallisation in the dried samples was not supported by their NMR spectra.

The large difference in melting points,[1, 6, 7] however, were confirmed although no depression below the lowest melting compound within each pair was observed in mixed melting point determinations of rubixanthin–gazaniaxanthin and the corresponding acetates.

TABLE 4. COMPOSITION OF THE IODINE CATALYSED EQUILIBRIUM MIXTURES OF RUBIXANTHIN (*Rosa rubiginosa*) AND GAZANIAXANTHIN (*Gazania rigens*) AND THEIR ACETATES IN PETROLEUM ETHER

Carotenoid	Member of the stereoisomeric set	R_f-value kieselguhr paper		Acetone λ_{max} in nm	Relative ratio
		1%*	2%		
Rubixanthin	neo B		0·52	350 (429) 455 484	1
	neo A		0·45	350 (430) 457 485	2
	trans		0·39	435 462 492	7
Gazaniaxanthin	neo B		0·52	349 (428) 453 482	2
	neo A		0·45	349 (430) 455 483	2
	"*trans*"		0·39	435 462 492	6
Rubixanthin acetate	neo B	0·76		345 (430) 454 482	
	neo A	0·67		345 (430) 456 485	
	trans	0·62		435 462 492	
Gazaniaxanthin acetate	neo B	0·76		345 (429) 453 481	
	neo A	0·67		345 432 456 485	
	"*trans*"	0·62		436 462 492	

* Acetone in petroleum ether.

The chemical and physical evidence discussed so far reveal no structural differences between rubixanthin and gazaniaxanthin. The differences in melting point could, as already indicated by the extinction coefficient in visible light, be explained by an impurity in gazaniaxanthin. However, polymorphism or stereochemical differences were also considered.

The question of polymorphism of carotenoids, particularly of γ-carotene, has been discussed by various authors.[11, 12] The melting point of gazaniaxanthin remained unchanged after recrystallization initiated by seeding with a crystal of rubixanthin. Nor did the mixed melting point determination of the two alcohols support such an explanation.

Rubixanthin possesses one asymmetric carbon atom (C-3) only. It has been reported that rubixanthin is optically inactive ($\alpha_{\mathrm{Cd}}^{20} = \pm 10$ in benzene)[1] and that gazaniaxanthin exhibits no rotation in benzene or petroleum ether and moderate rotation ($\alpha_{\mathrm{Cd}}^{25} = +65$)[7] in chloroform. CD-measurements (Fig. 2) were carried out. Both rubixanthin, contrary to the previous report,[1] and gazaniaxanthin were found to be optically active. The ORD- (of which only preliminary measurements were made) and the CD-curves of the two substances were qualitatively very similar, gazaniaxanthin showing the more intense optical activity (rotary strengths) of the two. Provided the hydroxyl group was located at the same carbon atom in the two compounds, this part of the molecule appeared to have the same absolute configuration.

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[11] L. ZECHMEISTER and W. A. SCHROEDER, *Arch. Biochem.* **1**, 231 (1942).

[12] R. RÜEGG, U. SCHWIETER, G. RYSER, P. SCHUDEL and O. ISLER, *Helv. Chim. Acta* **44**, 985 (1961).

The melting point differences for rubixanthin and gazaniaxanthin could thus not be explained merely by stereochemical differences at C-3. Moreover, the visible spectra and *cis–trans* isomerization studies did not favour the presence of *cis* double bonds in either compound.[13]

At this stage Brown and Weedon [14] in a short communication reported in part parallel experiments and results, which prompts us to publish our data. They concluded that rubixanthin and gazaniaxanthin both had structure I, rubixanthin being the all-*trans* form and gazaniaxanthin the isomer with a *cis*-configuration about the 5′,6′ double bond. This conclusion was strongly supported by the fact that the melting point of gazaniaxanthin could be raised to that of rubixanthin after iodine catalysed isomerization and chromatography. Their conclusion is consistent with the results obtained by us, including the two melting regions of gazaniaxanthin acetate (Table 2). Except for the inconclusive statement that a weak M-18 peak in the mass spectra of the two compounds was compatible with a 3-hydroxy end-group of the zeaxanthin type, Brown and Weedon[14] did not discuss the location of the hydroxyl group.

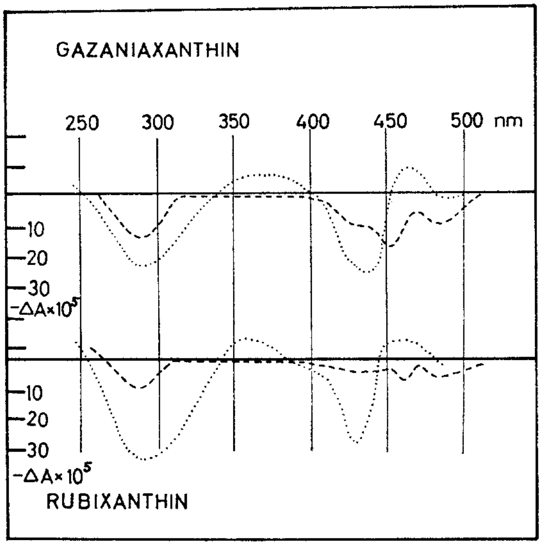

FIG. 2. MEASURED CIRCULAR DICHROISM OF 1 cm LAYER OF SOLUTIONS OF GAZANIAXANTHIN AND AND RUBIXANTHIN IN CCl_4 (.....) AND IN *n*-HEXANE (– – –).

The absorbance in the maximum at 460 nm of the *n*-hexane solutions (1 cm light path) was for gazaniaxanthin 2·00 ($\sim 1{\cdot}3 \times 10^{-5}$ mole $l.^{-1}$) and for rubixanthin 3·72 ($\sim 2{\cdot}2 \times 10^{-5}$ mole $l.^{-1}$). The molar CD in the extremum at *ca.* 455 nm is thus for gazaniaxanthin *ca.* 13 and for rubixanthin *ca.* 2·7.

EXPERIMENTAL

Materials and General Methods

When not specified, these were as summarized elsewhere.[15] NMR spectra were recorded on a Varian A-60-A (60 Mc/sec) spectrometer and mass spectra on an LKB-9000 instrument as reported elsewhere.[10] CD measurements were recorded on a Roussel-Jouan Dichrographe with increased sensitivity.

The physical properties of the compounds studied are summarized in Tables 1–4 and Figs. 1–2. Column chromatography was performed on neutral alumina, activity grade 2.

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[13] L. ZECHMEISTER, *cis–trans Isomeric Carotenoids, Vitamins A and Arylpolyenes*, p. 28, Springer, Wien (1962).

[14] B. O. BROWN and B. C. L. WEEDON, *Chem. Commun.* 382 (1968).

[15] A. J. AASEN and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **20**, 1970 (1966).

Rubixanthin

Frozen peels of *Rosa rubiginosa* (1 kg), collected at Norway Institute of Technology in September 1967, were dehydrated with methanol in a Waring blendor and the pigments extracted with acetone. The carotenoids were transferred to ether in the usual manner and submitted to column chromatography. Rubixanthin natural ester was saponified and rubixanthin purified by column chromatography, crystallization from acetone–methanol and recrystallization from benzene–methanol (2:5), yield 19 mg. Rubixanthin (40 mg) was acetylated and rubixanthin acetate purified by column chromatography and recrystallization from benzene–methanol, yield 18 mg. Oxidation of rubixanthin (0·4 mg) with *p*-chloranil gave no products with extended chromophore.

Gazaniaxanthin

Gazaniaxanthin was partly isolated from dried flowers of *Gazania rigens* (63 g) collected in Sandringham, Victoria, Australia, in March 1966, by acetone extraction. The ester present was saponified and gazaniaxanthin repeatedly chromatographed on deactivated alumina. White contaminants were removed by partial crystallization from cold acetone. The acetate was prepared in the usual manner. Gazaniaxanthin from the Australian source exhibited visible spectrum, R_f-value and i.r. spectrum identical with gazaniaxanthin isolated by Zechmeister and Schroeder.[7] M.p.s were in the same range (Table 2) and the acetates agreed in their visible light spectra and R_f-values. The m.p. of gazaniaxanthin was not changed by recrystallization, acetylation and saponification (employing column chromatography at each stage) nor by priming the mother liquor with a crystal of rubixanthin. Oxidation of gazaniaxanthin with *p*-chloranil gave no allylic oxidation products.

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