Hydrophilic Carotenoids: Surface Properties and Aggregation Behavior of the Potassium Salt of the Highly Unsaturated Diacid Norbixin

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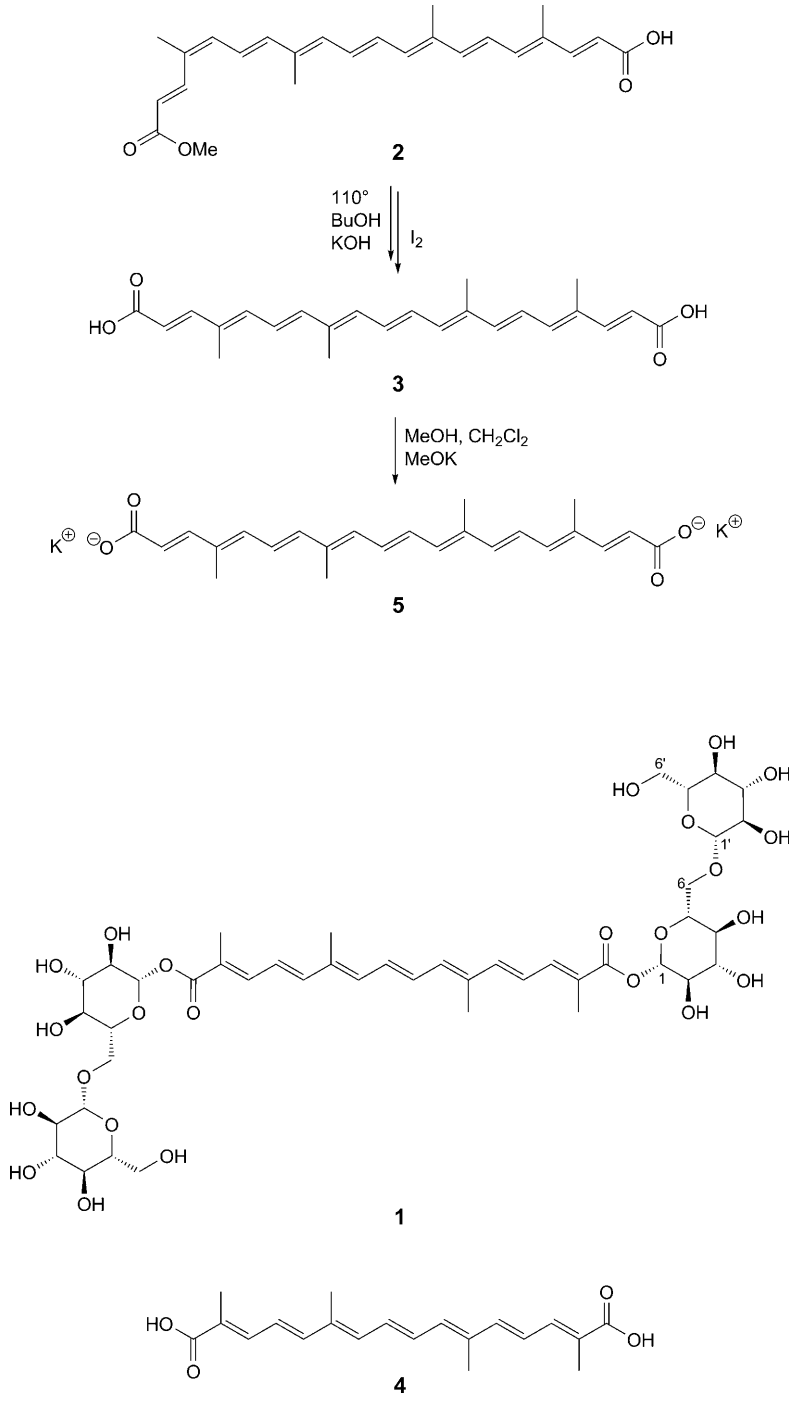
The oft-claimed 'good' water solubility of the food color norbixin (**3**) could not be confirmed. In contrast, the potassium salt **5** of norbixin formed suitable dispersions. The surface and aggregation properties of salt **5** were investigated and compared with other naturally occurring and synthetic hydrophilic carotenoids (*Table*).

Introduction. – Considered in a historical perspective, two carotenoids out of the known *ca.* 750 have been abundantly used since ancient times: crocin (**1**) and bixin (**2**) [1–3]. These two carotenoids are highly unsaturated diacid derivatives: crocin (**1**) is a naturally occurring sugar ester, and bixin (**2**) (*Scheme*) is a monomethyl ester (crocin = bis(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-8,8′-diapo-ψ,ψ-carotenedioate = 6-*O*-β-D-glucopyranosyl-β-D-glucopyranose 1,1′-[(2*E*,4*E*,6*E*,8*E*,10*E*,12*E*,14*E*)-2,6,11,15-tetramethylhexadeca-2,4,6,8,10,12,14-heptaenedioate]; bixin = 6-methyl 6′-hydrogen 9-*cis*-6,6′-diapo-ψ,ψ-carotenedioate = 1-methyl 20-hydrogen (2*E*,4*Z*,6*E*,8*E*,10*E*,12*E*,14*E*,16*E*,18*E*)-4,8,13,17-tetramethyleicosa-2,4,6,8,10,12,14,16,18-nonaenedioate). Both compounds are frequently used as food colors. Crocin (**1**) is highly soluble in H_2O; there is practically no saturation point [4]. Bixin (**2**) is H_2O insoluble. Hydrolyzing bixin (**2**) gives norbixin (=6,6′-diapo-ψ,ψ-carotenedioic acid; **3**), which is often presented in the literature as H_2O-soluble, *e.g.*, it is claimed that solutions up to 5% can be achieved [5–8]. Crocin (**1**) and norbixin (**3**) are therefore often identified as the two outstanding H_2O-soluble compounds in the range of the otherwise hydrophobic carotenoids. However, there is no plausible reason for claiming that the longer-chain diacid norbixin (**3**; C_{24}) is H_2O soluble, when the shorter-chain crocetin (**4**; C_{20}) has been found to be nearly insoluble in H_2O [9]. Consequently, other authors are more definitive and affirm that the Na or K salts of norbixin are the true H_2O-soluble compounds [2][10].

The dipotassium salt **5** of norbixin is composed of a hydrophobic polyene chain connected to hydrophilic groups at both ends. This structural characteristic confers to the molecule the character of a bolaamphiphile, showing surfactant activity and the tendency to form aggregates in H_2O. Besides its use as food color (E160b), norbixin (**3**) and its Na or K salts (see **5**) react as antioxidants and singlet-oxygen quenchers, and demonstrate other biological activities [7][11–13].

In spite of the widespread commercial applications of norbixin and its salts, the surfactant data and aggregation properties of these biodegradable bolaamphiphiles has

Scheme


OH
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2
110°
BuOH
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not been investigated. The now presented results are an extension of our investigations of natural and synthetic hydrophilic carotenoids [4][14–16].

Results and Discussion. – *Synthesis of Norbixin* (**3**). Bixin (**2**) was hydrolyzed in BuOH with 25% KOH/MeOH at 110° for 7 h. Although high temperature favors isomerization to the more stable all-*trans*-configurated norbixin (**3**), a mixture of **3** and its *cis*-isomer was obtained (VIS spectrum: *cis*-peak at 350 nm) [17]. Since it appeared difficult to separate these isomers by column chromatography, the mixture was isomerized by adding some drops of a methanolic I_2 solution [18]. After workup and freeze-drying, pure *trans*-configurated **3** was collected (*Scheme*).

Aggregation Behavior of Norbixin (**3**). A few drops of MeOH were given to norbixin (**3**) prior to adding H_2O to facilitate dispersion formation. After stirring one week under N_2 at 20°, the dispersibility of **3** was determined spectroscopically to 0.2%. It was found that **3** appeared predominantly as *H*-aggregates [19] in H_2O, which strongly suggests that even at this low concentration, an aggregate dispersion is present and not, as has been stated, a solution. The critical H_2O concentration in MeOH for aggregation is 62% (*Fig. 1*). Large aggregates were formed with a hydrodynamic radius $r_H \approx 2.3$ μm. The low hydrophilicity of norbixin (**3**) prevented tensiometric measurements. The hydrophilicity, however, was high enough to inhibit the preparation of surface monolayers [20].

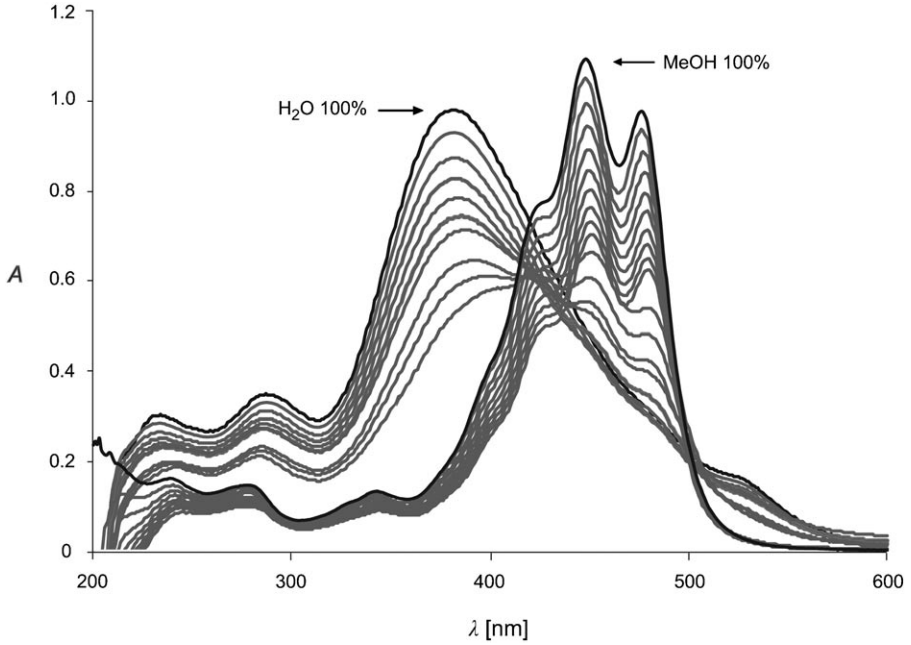

Fig. 1. *UV/VIS Spectra of norbixin* (**3**) *as a function of solvent concentration.* In H_2O λ_{max} 384 nm, in MeOH λ_{max} 450 nm. With gradual addition of H_2O to the MeOH solution, aggregates are formed; in contrast, with gradual addition of MeOH to the aqueous dispersion, the aggregates are disrupted. The critical solvent concentration for aggregation is 62% H_2O in MeOH; for disruption, 38% MeOH in H_2O.

Surface Tension and Critical Aggregate Concentration of the Potassium Salt **5** *of Norbixin* (**3**). Since the hydrophilicity of acid **3** is too low for practical purposes, potassium salt **5** of norbixin was prepared. Salt **5** was dispersible in H_2O to 8% and formed *H*-aggregates (*Fig. 2*) with $r_H \approx 50$ nm. Rotational peaks in the intensity plot of the particle-size analyzer indicated the occurrence of nonspherical aggregates. The surface tension γ for various concentrations (c) of **5** in H_2O was determined with a tensiometer. A plot of γ *vs.* ln c gave, at the point of discontinuity, the critical aggregate concentration $c_M = 0.0004$ mol/l ($= 0.16$ mg/ml $\approx 0.02\%$); at this point, the surface tension was $\gamma_{c_M} = 56$ mN/m (*Table*). The surface-tension measurements were preceded by long equilibrium periods (> 20 min) each time the γ of a new concentration was measured (*Fig. 3*). The values were recorded after 25 min and are, therefore, considered to be indicative rather than definite.

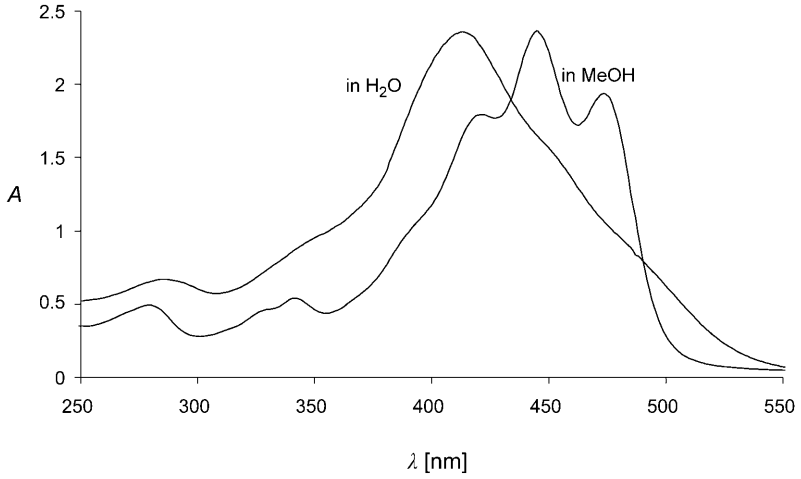

Fig. 2. *UV/VIS Spectra of potassium salt* **5** *of norbixin in* H_2O (λ_{max} 415 nm) *and MeOH* (λ_{max} 445 nm)

Table. *Selected Data for Potassium Salt* **5** *of Norbixin* (**3**) *and Related Compounds*

	Norbixin (**3**)	Potassium salt **5**	Crocin (**1**) [4]	*Cardax*™[a])	Astalysine[b])
Sol./disp. [%]	0.2	8	no saturation	0.9	18
γ_{c_M} [mN/m]		56	52	60	58
π_{c_M} [mN/m]		16	21	13	14
c_M [mM]		0.36	0.82	0.45	2.18
$\Gamma\ 10^{-6}$ [mol | m^2]		0.73	1.4	0.7	0.7
a_m [Å^2]		230	115	240	240
ΔG_{ag} [kJ/mol]		– 59	– 17.5	– 54.8	– 90.4
ΔG_{ad} [kJ/mol]		– 82	– 32.5	– 73.6	– 109.7
ΔG_{ad-ag} [kJ/mol]		– 23	– 15	– 18.8	– 19.3
K_M		2900	1200	1800	1500
K_{ad}		61000	450000	23000	7200
K_{ad-ag}		22	370	13	5
AMER		1.4	1.8	1.3	1.2

[a]) Data taken from [15]. [b]) Data taken from [16].

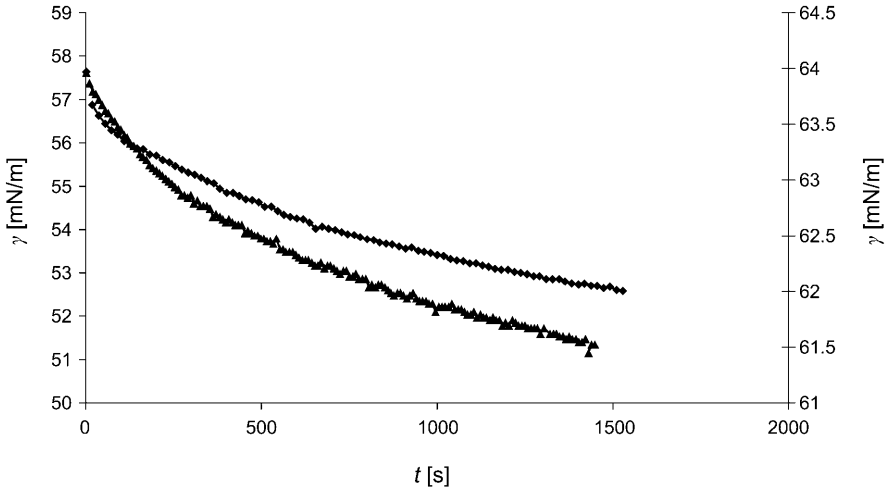

Fig. 3. *Change of surface tension γ for potassium salt* **5** *of norbixin in H_2O with time.* ▲, $c = 98$ mg/l (left axis); ◆, $c = 1000$ mg/l (right axis).

The surface excess concentration Γ was calculated with the equation $\Gamma = (-1/nRT) \cdot (d\gamma/d(\ln c)) = (-c/nRT) \cdot (d\gamma/dc)$ with $n = 3$, assuming full dissociation of the salt in H_2O. The measured low surface concentration $\Gamma = 0.7 \cdot 10^{-6}$ mol/m^2 corresponds to a high molecule area $a_m = 230$ Å^2, which can be associated with horizontally lying molecules of **5** at the water surface with both end groups anchored in the H_2O. Other ionic bolaamphiphilic carotenoids behave similarly (*Table*). With the values of c_M, γ, Γ, a_m, and the surface pressure πc_M, the free energy of adsorption ΔG°_{ad} and aggregation ΔG°_{ag} as well as the equilibrium constants for aggregation and surface adsorption were calculated. ΔG°_{ag} was derived from the equation $\Delta G^\circ_{ag} = nRT \ln c_M + 2RT\beta \ln 2$ [21]. Again, the prefactor n denotes the number of dissociated species in H_2O – similar to other carotenoid salts with low c_M (*Table*), we assumed full dissociation of **5** and set $n = 3$ [15][16]. The free energy of adsorption was calculated with $\Delta G^\circ_{ad} = \Delta G^\circ_{ag} - 6.023 \cdot 10^{-3}$. The equilibrium constants reflect the high preference of the molecules of **5** to be adsorbed at the water surface. The adsorption-micellar energy ratio (AMER) [22] $\Delta G^\circ_{ad}/\Delta G^\circ_{ag}$ has been proposed as a surfactant-performance indicator. AMER Values close to unity imply dense monolayer formation, enhanced micelle concentration, and high ability in flotation, cleaning, and wetting. The AMER value for salt **5** is similar to other ionic carotenoid surfactants.

Conclusion. – The potassium salt **5** of norbixin in H_2O formed clear yellow dispersions and showed the typical surface and aggregation properties of (ionic) carotenoid bolaamphiphiles. Norbixin (**3**) was found to be almost insoluble (as well as indispersible) in H_2O, in contrast to previous statements in the literature. Crocin (**1**) remains as the sole naturally – and abundantly occurring – carotenoid exhibiting true H_2O solubility.

Experimental Part

1. *Norbixin* (**3**). Bixin (**2**) was hydrolyzed by modifying a previous procedure [23]. Thus, **2** (0.1 g, 0.2535 mmol) was dissolved in 25% KOH/MeOH (0.25 ml), and BuOH (5 ml) was added. The soln. was heated to 110° under N_2 for 7 h (TLC monitoring (silica gel sheets, acetone/hexane 1.5 : 1)). After solvent evaporation, H_2SO_4 (1.25 ml, 25%) was added and the soln. stirred for 5 min. Then, H_2O (5 ml) was added, and the mixture stirred for 30 min at r.t. CH_2Cl_2 (5 ml) was added, and the org. phase washed several times with H_2O until neutrality and then concentrated. The product was dissolved in acetone and dried (Na_2SO_4). TLC and subsequent VIS-spectra analysis showed the occurrence of a *cis*-isomer (*cis*-peak at 350 nm). The isomers were dissolved in MeOH and several drops of a I_2/MeOH soln. were added, which catalyzed *cis* to *trans* isomerization. After freeze-drying, **3** (29 mg, 30%) was obtained. VIS: *Fig. 1*. ^{1}H-NMR (400 MHz, CD_3OD): 7.85 (*d*, HC=C*H*COOH); 7.3 – 6.2 (*m*, CH of the polyene); 6.1 – 5.8 (*m*, C*H*=CH–COOH); 2.1 – 1.9 (4 Me) [7]; no signals for the *cis*-isomer and for MeO of bixin (**2**). ESI-MS: 403 ($[380 + Na]^+$) [17].

2. *Norbixin Dipotassium Salt* (*=(2*E*,4*E*,6*E*,8*E*,10*E*,12*E*,14*E*,16*E*,18*E*)-4,8,13,17-Tetramethyleicosa-2,4,6,8,10,12,14,16,18-nonaenedioic Acid Potassium Salt (1:2)*; **5**). Norbixin (**3**; 40.5 mg, 0.1064 mmol) was dissolved in CH_2Cl_2/MeOH 1 : 0.6 (60 ml) at 0°, and the same mol amount of 25% MeOK/MeOH (63 ml, 0.2134 mmol) for each COOH group was added. The solvents were evaporated without heating. VIS: *Fig. 2*.

3. *Aggregation*. Aggregate behavior was monitored by dispersing a known amount of **3** with H_2O in a UV cell. Several µl of MeOH were added prior to adding H_2O to assure full dispersion. MeOH quantities (150 µl) were continuously added, and the spectra recorded until the aggregates were disrupted forming a monomolecular soln. Similarly, to a specified amount of **3** dissolved in MeOH, H_2O (150 µl) was gradually added until the aggregation peak was observed. Both measurements gave similar results (*Fig. 1*).

4. *Dispersibility.* Dispersibility of **3** and **5** was determined spectroscopically in H_2O (*Milli-Q*).

5. *Particle Size*. Aggregate size was determined with a *N5* submicron-particle-size analyzer (by PCS; *Beckman Coulter, Inc.*, Fullerton) at angles presenting reliable values after filtering the dispersion with a 200-nm filter.

6. *Surface Parameters.* Critical aggregate concentration and surface tension were determined in a conical, *Teflon*-coated vessel with a *Wilhelmy* plate on a *Krüss-K100* tensiometer by gradually adding H_2O to an aq. dispersion of **5** with a *Metrohm-765* dosimat. The γ value was taken 25 min after each dilution (*Fig. 3*). The measurements were made in duplicate at different times by different operators. For the calculation of thermodynamic data, see [4].

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