The Carotenoids of Two *Juncus spp.* (Family Juncaceae) and One *Scirpus sp.* (Family Cyperaceae)

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The carotenoids of *Juncus gerardi* Lois., *J. bufonius* L. (Table 1), and of *Scirpus silvaticus* L. (Table 2) have been investigated by chromatographic separation. Lutein and β-carotene are the dominating carotenoids of these three species and seem to comprise between 80 and 90 % of the total carotenoid content.

Table 1. Carotenoids of *Juncus gerardi* Lois. and *J. bufonius* L. Zones numbered in order of decreasing adsorptivity.

Zone No.	Colour	Absorption maxima, mμ. Solvent benzene		
1	Pale yellow	433	408	386
2	Pale orange	475	446	422
3[a]	Yellow	458	431	408
4	Yellow	459	433	408
5	Orange	482	454	428
6[a]	Orange	482	453	426
7	Orange	487	456	428
		Solvent petroleum ether		
8[a]	Orange	473	446	422
9	Yellow	448	422	400
10[a]	Orange	471	443	420
11	Orange	478	450	
12	Orange	474	446	
13	Colourless	368	346	333

[a] Not observed in *J. bufonius*.

cis-Isomers of lutein and β-carotene were not included in the tables. For literature values, *cf.* Ref. 1.

β-Carotene was obtained in a crystalline state from fraction 11, while fraction 12 seemed to contain α-carotene. Fraction 13 gave an absorption spectrum typical for phytofluene.

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Fraction 7 was chromatographically and spectroscopically identical with an authentic sample of lutein, while fraction 8 displayed the spectrum of cryptoxanthin, and also the typical behaviour of cryptoxanthin when distributed between petroleum ether and 90/95 % methanol.

The remaining zones were very weak, compared with the lutein and β-carotene zones. The substances isolated from these zones gave a blue colour when treated with hydrochloric acid in ethyl ether, demonstrating the presence of epoxy groups.

Fraction 1 displayed an auroxanthin-type spectrum. Fraction 2 gave a spectrum corresponding to that of neoxanthin; when dissolved in methanol this substance absorbed at 467, 439, and 416 mμ, and acid treatment gave a hypsochromic shift of 17 mμ, as expected for the formation of neoneoxanthin.[2] The fractions 3 and 4 gave spectra of the flavoxanthin/chrysanthemaxanthin type. Fraction 5 seemed to contain violaxanthin, since it gave a substance of the auroxanthin type upon acid treatment. Fraction 6 displayed the spectrum of lutein epoxide; acid treatment gave a substance which absorbed at 458, 431, and 406 mμ.

Fraction 9 was found in both *J. gerardi* and *J. bufonius*. The zones appeared somewhat above the β-carotene zones in the chromatograms of the epiphasic carotenoids. The substances were relatively stable towards acids, but gave a distinct colour with hydrochloric acid in ethyl ether. These properties suggest the presence of flavochrome (α-carotene-5,8-epoxide), which is supported by the occurrence of a faint fraction 10 in the *J. gerardi* chromatogram,

Table 2. Carotenoids of *Scirpus silvaticus* L.

Zone No.	Colour	Absorption maxima, mμ. Solvent benzene		
1	Pale orange	Mixed zones		
2	Yellow	458	429	406
3	Yellow	459	430	407
4	Orange	479	452	428
5	Orange	485	455	430
		Solvent petroleum ether		
6	Yellow	449	423	400
7	Orange	470	441	418
8	Orange	476	448	
9	Orange	473	445	

which spectroscopically corresponded to α-carotene-5,6-epoxide.

The carotenoids of *S. silvaticus* corresponded well to those of the two *Juncus* species.

Fraction 1 seemed to consist of a mixture of auroxanthin and neoxanthin, but was too small for further separation. The fractions 2 and 3 displayed spectra of the flavoxanthin/chrysanthemaxanthin type, but were considerably stronger than in the *Juncus* chromatograms. Fraction 4 gave a spectrum corresponding to violaxanthin and formed an auroxanthin-type substance upon acid treatment.

In this chromatogram the fractions 5, 8, and 9 were spectroscopically and chromatographically identical with the corresponding fractions from *Juncus* (lutein, β-carotene, and α-carotene, respectively). The epoxidic fractions 6 and 7 seemed to be identical to fractions 9 and 10 in Table 1.

In *Juncus gerardi* some fractions were found, which were not observed in the other plants. In most cases these zones were very faint, and corresponding zones in the other chromatograms may have escaped identification.

The steroids of the three species investigated, and also of *Luzula pilosa* (L.) Willd. (Juncaceae) and of *Carex echinata* Murr. (Cyperaceae), were identified by means of gas chromatography. The presence of β-sitosterol was demonstrated in all species. From *J. gerardi* and *L. pilosa* this substance was furthermore isolated in a crystalline state. It gave an infrared spectrum identical with that of an authentic sample. All gas chromatograms also showed a peak corresponding to stigmasterol, which was, however, present in considerably lower concentrations than β-sitosterol. No other peaks were observed.

Experimental. Carotenoids: Fresh plant material of *Juncus gerardi* Lois. (collected at Straumsnes, Norway), *J. bufonius* L., and *Scirpus silvaticus* L. (both collected at Ås, Norway) was extracted with acetone in a nitrogen atmosphere. The extracts were concentrated, and the carotenoids transferred to peroxide free ether. After saponification with methanolic potassium hydroxide, the carotenoids were partitioned between petroleum ether and 90 % methanol. The hypophasic fractions were chromatographed on columns of precipitated calcium carbonate (Riedel-de Haën) and developed with benzene. The epiphasic fractions were chromatographed on columns of calcium hydroxide and developed with petroleum ether. The spectra were measured on a Beckman DB recording spectrophotometer. Most fractions were rechromatographed on kieselguhr-containing paper (xanthophylls)[3] or aluminium oxide-containing paper (carotenes),[4] and the spectra were measured again after the substances were purified in this way.

Sterols: After saponification of the ether extracts, the total unsaponifiable fractions were dissolved in acetone. Sterols and fatty alcohols were precipitated by cooling to −60°C. The carotenoids were worked up as above, while the sterols were isolated by digitonin precipitation.[5] After recovery of the sterols from their digitonides, they were trimethylsilylated and subjected to gas chromatography. β-Sitosterol (m.p. 139°), was isolated from *Juncus gerardi* and *Luzula pilosa* by means of chromatography on columns of activated alumina, while stigmasterol was not obtained in a pure state.

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1. Karrer, P. and Jucker, E. *Carotinoide*, Birkhäuser, Basel 1948.
2. Curl, A. L. and Bailey, G. F. *Food Research* **22** (1957) 323.
3. Jensen, A. and Liaaen Jensen, S. *Acta Chem. Scand.* **13** (1959) 1863.
4. Jensen, A. *Acta Chem. Scand.* **14** (1960) 2051.
5. Sperry, W. M. *J. Lipid Res.* **4** (1963) 221.

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