CH_3OH: C 75.0; H 10.78. Found, after drying: C 77.2; H 10.64. Calc. for $C_{27}H_{44}O_3$: C 77.8; H 10.65.)

The acetylated sapogenin, after recrystallisation from methanol, melted at 128 −30°. $[\alpha]_D^{20}$ −67°. (Found: C 73.2; H 10.21. Calc. for $C_{29}H_{46}O_4 + CH_3OH$: C 73.4; H 10.27. When dried by melting in vacuum, the acetate had m.p. 142−44°. (Found: C 76.2; H 10.19. Calc. for $C_{29}H_{46}O_4$: C 75.9; H 10.11.)

The chemical and physical properties of the substance are close to those of sarsasapogenin. The melting point of the sapogenin showed no depression on admixture with an authentic sample of sarsasapogenin. The identity of the two substances was further confirmed by the infrared spectra.

I wish to express my thanks to Professor N. A. Sörensen for his kind interest, and for determining the I. R. spectra.

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The Carotenoids of *Shepherdia canadensis* (L.) Nutt.

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Through the courtesy of Mr. Olav Gjærevoll, Museum of the Royal Norwegian Society of Sciences, Trondheim, a small sample of the fruits of *Shepherdia canadensis* has been obtained. The fruits were collected by Mr. Gjærevoll in Alaska, where they are called "soap berries". They are eaten by the natives, after being whipped with sugar to a fluffy mass.

An aqueous extract of the fruits indeed gave an extremely rich and stable foam on shaking. The fruits had a moderately bitter taste. Work on isolation of the saponin, and, if possible, of the bitter principle will be attempted.

The fruits were intensely red in colour, due to substances of carotenoid nature, and it was considered worth while first to examine these substances. The carotenoids of other members of the *Elaeagnaceae* have already been subjected to such investigation. From the fruits of the sea buckthorn, (*Hippophaë rhamnoides* L.) zeaxanthin (Karrer and Wehrli [1]) and physaliene (Winterstein and Ehrenberg [2]) have been isolated. More recently cryptoxanthin, lycopene, β-carotene and γ-carotene have been added to the list by Bielig [3]. In *Elaeagnus longipes* Gray, lycoxanthin, lycopene and γ-carotene have been found by Geiger-Vifian and Müller [4].

By chromatographic separation of the carotenoids of *Shepherdia canadensis* the fractions shown in Table 1 were obtained.

Table 1.

Zone No.	Colour	Absorption maxima, mμ, solvent petroleum ether		
1	Colourless	365	346	330
2	Diffuse khaki	475	447	425
3	Pale yellow	425	400	379
4	Khaki	477	448	
5	Pale yellow	425	399	378
6	*Yellow-orange*	*485*	*455*	*432*
7	Yellow	466	437	414
8	Yellow-orange	485	456	432
9	*Orange*	*496*	*462*	*434*
10	*Red*	*504*	*473*	*444*
11	*Dark red*	*503*	*473*	*445*
12	Salmon	485	452	
13	Pale yellow	425	399	378
14	Pale salmon	472	443	420
15	Yellow	448	425	400
16	Mauve	490	455	430
17	Salmon	Single max. at 450		
18	Yellow	450	421	400
19	Yellow	450	422	400

The zones are numbered in order of increasing absorbtive power. The zones 1−11 were chromatographed on calcium hydroxide, the other zones on calcium carbonate.

The fractions 6 and 9−11 (printed in italics), contained the main bulk of the carotenoids. The fractions 12−19 contained very small amounts of pigments.

Fraction 11 gave after several recrystallisations from benzene-methanol dark, bluish

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red crystals which melted sharply at 137°corr. After saponification the substance melted at 162°corr. The absorption maxima corresponded closely to the values for lycoxanthin, whose m.p. is 164°, according to Zechmeister and Cholnoky [5]. These authors have synthesised lycoxanthin acetate. Its m.p., 137°, is the same as that of the substance isolated from *Shepherdia*. Since esters with higher fatty acids would be expected to have lower melting points, the identity of the isolated substance with lycoxanthin acetate seems very probable.

The neighbouring zone, fraction 10, was chromatographically identical with lycopene from tomatoes. Fraction 9 was not obtained in a crystalline form. After repeated chromatographic separation it seemed to contain no traces of other pigments and gave the absorption bands of γ-carotene [6]. However, in contrast to γ-carotene, the substance had two fairly pronounced peaks at 353 and 369 mμ.

No definite conclusions can be reached with respect to the identity of the other carotenoids. The fractions 1 and 4 displayed absorption maxima very similar to those of phytofluene [7], and α-carotene [8], respectively.

The pale yellow zones 3 and 5 showed the absorption bands of η-carotene [9] or ζ-carotene [10]. The orange zones 6 and 8 both displayed the spectrum of β-carotene.

The fractions 12—14 were epiphasic, but on saponification they turned hypophasic, consequently the pigments were xanthophyll esters. The absorption spectra of fractions 18 and 19 corresponded closely to chrysanthemaxanthin [11] and flavoxanthin [12]. The latter, when dissolved in ether, gave a weak blue colour with hydrochloric acid.

No attempt was made to correlate the zones 2, 7, and 15—17 with known caroteoids.

Experimental. Ripe fruits of *Shepherdia canadensis* were collected at Beaver Creek, White Mountains, Alaska, during the summer of 1953. Sugar was added as a preservative, the mixture heated until boiling, and stored in a 1 lb. glass jar until used.

The fruits were washed with water, treated with acetone to extract the carotenoids, and the carotenoids transferred to petroleum ether (b. r. 40—70°) by addition of water. The petroleum ether extract was washed with water and dried over anhydrous sodium sulphate. All operations were carried out in an atmosphere of pure nitrogen. After evaporation to dryness at 30°, the carotenoids were redissolved in petroleum ether and chromatographed on a column (2 × 20 cm) of precipitated calcium carbonate (Riedel-de Haën). The column was developed with petroleum ether containing a trace of acetone until zone 11 had just left the column. Each of the zones 12—19 were cut out separately and their absorption spectra measured in petroleum ether solution with a Beckmann DU spectrophotometer.

The eluate from the column was collected in two fractions; the first fraction contained the zones 1—8, and the second fraction the zones 9—11. The latter was rechromatographed on a column of calcium hydroxide (Riedel-de Haën) and developed with petroleum ether, containing just enough acetone to cause a slow downward movement of the pigment bands. Each zone was rechromatographed separately until homogeneity, and the eluted substances dissolved in 1 ml benzene. Upon careful addition of methanol the pigments from the zones 10 and 11 crystallised.

Also the first fraction of the eluate, containing the zones 1—8, was chromatographed on calcium hydroxide, and the absorption spectra recorded.

I wish to express my thanks to Professor N. A. Sörensen for his kind interest in the work.

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