chrome *c* concentration was about the same.

Discussion. Chaffee *et al.*[8] using brown fat of hamsters, found that the mass as well as the total mitochondrial content increased during cold acclimatization. Schollmeyer and Klingenberg [9] have shown that the cytochrome *c* content of mitochondria from different organs and species differs very little. Stratmann and Hohorst,[6] however, found that cold adaptation of newborn guinea-pigs did not markedly influence the cytochrome concentration of the mitochondria of the brown adipose tissue but rearing the animals 20 days at + 20°C caused a decrease of the cytochromes. Studies are now in progress to investigate the "mitochondrial density" of the brown adipose tissue of hedgehogs during a period of one year. The above results do not give information on the dependence of the variations on the light-dark and temperature cycles or other environmental factors.

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"Asterinsäure" — an Acetylenic Carotenoid

N. A. SØRENSEN, S. LIAAEN JENSEN, B. BØRDALEN,[a] A. HAUG[b]

Organic Chemistry Laboratories, Norway Institute of Technology, Trondheim, Norway

C. ENZELL and G. FRANCIS

Research Department, Swedish Tobacco Company, Stockholm, Sweden

"Asterinsäure", first isolated by von Euler and Hellström [1] from the starfish *Asterias rubens*, exhibits properties reminescent of astacene (*1*) [2] or astaxanthin (*2*).[3] Karrer and Rübel [4,5] later isolated a compound considered to be astacene (*1*) from the same source. The conversion of astaxanthin (*2*) to astacene (*1*) by alkali in the presence of oxygen has been demonstrated by Kuhn and Sørensen.[3]

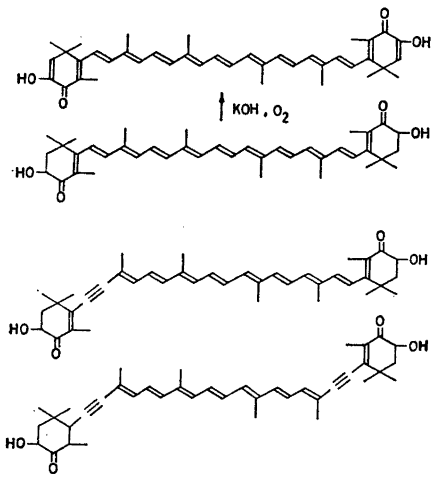
Twenty years ago "asterinsäure" was isolated from the back skin of *Asterias rubens* by two of us (B.B. and A.H.) following in principle the procedure of von Euler and Hellström [1] involving precipitation of the chromoprotein with ammonium sulphate, cleavage of the chromoprotein with alcohol, partition, and finally crystallization from pyridine-water.

The compound isolated has been reexamined in recent years, and preliminary results were considered to indicate identity with astaxanthin (*2*).[6a] However, the evidence presented here shows that these compounds are different and that "asterinsäure" (*3*) is an acetylenic analogue of astaxanthin (*2*), either 7,8-didehydro-astaxanthin (*3a*) or a mixture of *3a* and the diacetylenic derivative 7,8,7′,8′-tetradehydro-astaxanthin (*3b*).

Bluish needles of *3* were obtained from pyridine-water (*2* crystallizes as plates from the same solvent pair [6b]); yield 0.88 mg, m.p. 215.5–216°C in evacuated tube (reported for "asterinsäure" m.p. 185°C [1] and for 2 m.p. 215.5–216°C [3]).

When special precautions were taken to avoid crystallization on the paper *trans 2* and *3*

Present address: [a] Central Institute for Industrial Research, Oslo.
[b] Norwegian Institute of Seaweed Research, Trondheim.



were separated on kieselguhr paper (R_F = 0.46 and 0.28) and on calcium carbonate paper [7] (R_F = 0.34 and 0.27, respectively) using 10 % acetone in petroleum ether as solvent.

In pyridine *3* exhibited λ_{max} at (478), 495 and (522) mμ (previously reported 480 mμ [8]) and in CS_2 it had λ_{max} at (480), 518 and 541 mμ, while *2* had λ_{max} at 493 mμ (previously reported fine-structure [3] is erroneous) in pyridine and at 503 mμ in CS_2 (Fig. 1).

The infrared spectrum (KBr) of *3* (Fig. 2) had ν_{max} 3400 (OH); 2940, 2850 (CH); 2150 (−C≡C−), 1660 (conj. C=O); 1550 (conj. double bonds); 1480, 1460 (CH_2); 1375, 1360 (CH_3, *gem.* CH_3), 1325, 1300, 1280, 1232, 1178, 1145, 1125, 1072, 1032, 1015; 985, 972, 953 (*trans* disubst. double bonds), 883; 832 (*trans* trisubst. double bonds), and 745 cm^{-1}. Authentic *2* had ν_{max} 3400 (OH); 2950, 2920, 2880 (CH); 1655 (conj. C=O); 1600, 1575, 1550 (conj. double bonds); 1465, 1440 (CH_2); 1382, 1360 (CH_3, *gem.* CH_3), 1310, 1275, 1225, 1190, 1175, 1142, 1122, 1088, 1068, 1020, 1005; 980, 970 (*trans* disubst. double bonds); 872; and 828 (*trans* trisubst. double bonds) cm^{-1}. The α-ketol *2* and the diosphenol *1* differ significantly in their carbonyl absorption.[9]

The infrared spectra of astaxanthin (*2*) and "asterinsäure" (*3*) reveal a close relationship between the two compounds. However, the characteristic acetylenic absorption is observed for *3* only. Mono- and di-acetylenic carotenoids with triple bonds in 7,8,7′,8′-positions of marine origin have recently been reported by Mallams *et al.*[10] and Campbell *et al.*[11]

The mass spectra of astaxanthin (*2*) and "asterinsäure" (*3*) are given in Fig. 3. The spectrum of *2* shows the molecular ion peak at *m/e* 596 and prominent peaks at *m/e* 504 and 490, which are due to the well established losses of 92 and 106 mass units in the fragmentation of carotenoids.[12-14] The peaks at *m/e* 580 and 564 can be associated with the loss of one and two oxygens from the molecular ion, but further work is needed to confirm this. The loss of two hydrogens from the molecular ion, a reaction common in carotenoids,[12,14] gives rise to the peak at *m/e* 594 and subsequent losses of 92 and 106 mass units account for the peaks at *m/e* 502 and 488.

The peak at *m/e* 594 in the spectrum of "asterinsäure" (*3*) may be ascribed to the molecular ion and the prominent peaks at *m/e* 502 and 488 (loss of 92 and 106 mass units) are in agreement with this. The three prominent

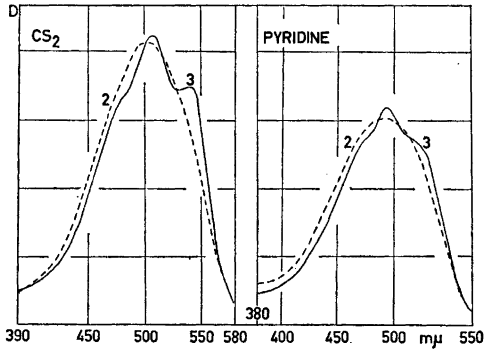


Fig. 1. Absorption spectra in visible light of *trans* astaxanthin (*2*) and *trans* "asterinsäure" (*3*) in carbon disulphide and in pyridine.

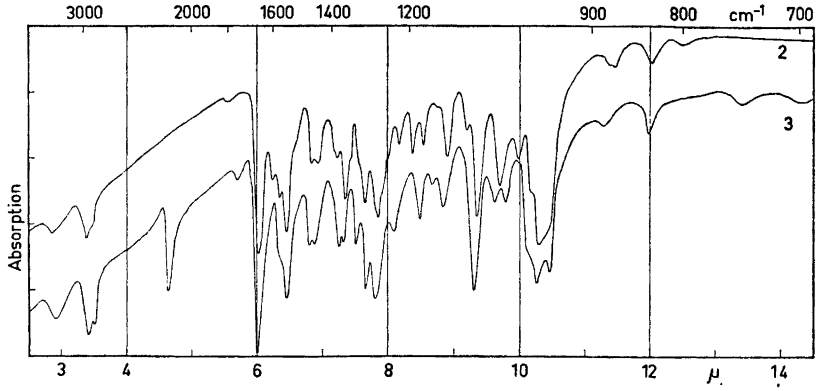


Fig. 2. Infrared spectra (KBr) of astaxanthin (*2*) and "asterinsäure" (*3*).

peaks at *m/e* 592, 500, and 486 occurring two mass units below the first set may be accounted for as described above, and the group of peaks centered at *m/e* 576 can be rationalized as dehydration or loss of oxygen from ions of higher molecular weight. These results indicate that the molecular weight of "asterinsäure" is two mass units less than that of astaxanthin and allow the former to be formulated as shown in structure *3a*.

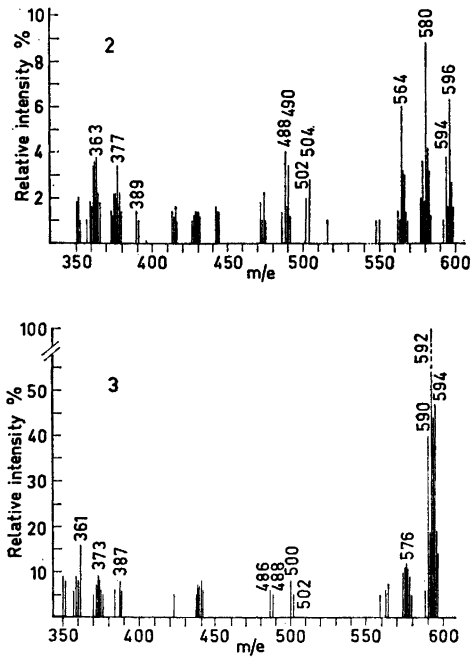


Fig. 3. Mass spectra of astaxanthin (*2*) and "asterinsäure" (*3*).

An alternative explanation of the mass spectral data is, however, possible. Although similar intensity ratios of M−2 to M peaks have been observed [14] in other carotenoid spectra it cannot on the basis of the mass-spectrometric evidence be excluded that the high intensity of the *m/e* 592 peak is due mainly to the molecular ion of the diacetylenic analogue *3b*.

Crystalline "asterinsäure" (*3*) gave a major zone (R_F = 0.28, *ca.* 95 %) and a minor zone (R_F = 0.31) on kieselguhr paper (10 % acetone in petroleum ether). It is not established whether these zones represented *3b* and *3a* or the *trans* and a *cis* isomer of *3a*.

On treatment with alkali *3* was converted to a product with acidic properties. Reduction of *3* with sodium borohydride gave a product with λ_{max} 452 and 481 mμ (diethyl ether) and spectral fine-structure indicating the presence of acetylenic bonds (see below). The peracetate of this presumed tetraol was somewhat more strongly adsorbed than authentic 3,4,3′,4′-tetraacetoxy-β-carotene. This evidence is in agreement with the ring structures *3a* or *3b* for "asterinsäure".

The assignment of the triple bond(s) to 7,8(7′,8′)-position is based on the relatively high intensity of the acetylenic infrared absorption, compared with that reported for alloxanthin,[10] which indicates a close proximity of the triple bond and the carbonyl function.[15] Moreover, the bathochromic shift in the visible light absorption spectrum of "asterinsäure" (*3*)

relative to that of astaxanthin (*2*) corresponds to that reported for the series alloxanthin (7,8,7′,8′-tetradehydro-zeaxanthin), diatoxanthin (7,8-didehydro-zeaxanthin) and zeaxanthin.[10,16] This can only be explained by assuming a corresponding location of the triple bond(s) in *3*. Triple bonds in other positions would result in a considerable hypsochromic shift.[17,18] The relatively marked fine-structure in the electronic spectra of diatoxanthin, alloxanthin,[16] and of "asterinsäure" (*3*) is believed to reflect the greater planarity of the chromophoric system in the acetylenic derivatives. Spectral fine-structure is generally observed for aliphatic polyenones or polyenals, but is virtually absent in the cyclic carotenones that lack acetylenic bonds in 7,8-position(s).

Isolation of "asterinsäure" for further experiments will be attempted. Authentic astaxanthin (*2*) for comparison was isolated by Sørensen and Stene from *Salmo trutta*.[19]

The experimental procedures used here have been summarized elsewhere.[20] Visible light absorption spectra were recorded on a Zeiss PMQ2 spectrophotometer. The mass spectra were obtained on an LKB-9000 mass spectrometer with direct inlet system under conditions specified elsewhere.[14]

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