chrome c concentration was about the same.

Discussion. Chaffee et al. using brown fat of hamsters, found that the mass as well as the total mitochondrial content increased during cold acclimatization. Schollmeyer and Klingenberg have shown that the cytochrome c content of mitochondria from different organs and species differs very little. Stratmann and Hohorst, 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

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"Asterinsäure", first isolated by von Euler and Hellström from the starfish Asterias rubens, exhibits properties reminiscent of astacene (1) or astaxanthin (2). Karrer and Rübel 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 Sörensen.

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 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 re-examined in recent years, and preliminary results were considered to indicate identity with astaxanthin (2). 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); yield 0.88 mg, m.p. 215.5–216°C in evacuated tube (reported for "asterinsäure" m.p. 185°C and for 2 m.p. 215.5–216°C). When special precautions were taken to avoid crystallization on the paper trans 2 and 3

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

In pyridine 3 exhibited \( \lambda_{\text{max}} \text{ at } (478), 495 \text{ and } (522) \text{ m}\mu \text{ (previously reported } 480 \text{ m}\mu) \) and in CS\(_2\) it had \( \lambda_{\text{max}} \text{ at } (480), 518 \text{ and } 541 \text{ m}\mu \text{, while 2 had } \lambda_{\text{max}} \text{ at } 493 \text{ m}\mu \text{ (previously reported fine-structure)} \) is erroneous in pyridine and at 503 m\( \mu \) in CS\(_2\) (Fig. 1).

The infrared spectrum (KBr) of 3 (Fig. 2) had \( \nu_{\text{max}} \text{ at } 3400 \text{ (OH)}; 2940, 2850 \text{ (CH)}; 1650 (\text{conjugated C=O}); 1550 (\text{conjugated double bonds}) \text{ and } 1375, 1380 \text{ (CH}_2\text{, gem. CH}_3) \text{ and } 1232, 1178, 1145, 1125, 1072, 1032, 1015; 985, 972, 953 \text{ cm}^{-1}. \) Authentic 2 had \( \nu_{\text{max}} \text{ at } 3400 \text{ (OH)}; 2950, 2920, 2880 \text{ (CH)}; 1655 (\text{conjugated C=O}) \text{ and } 1606, 1575, 1550 \text{ (conjugated double bonds)} \text{ and } 1465, 1440 \text{ (CH}_2) \text{ at } 1380, 1360 \text{ (CH}_2\text{, gem. CH}_3) \text{ at } 1310, 1275, 1225, 1190, 1175, 1142, 1122, 1088, 1068, 1020, 1005; 980, 970 \text{ (trans disubstituted double bonds)} \text{ and } 872 \text{ and } 828 \text{ (trans disubstituted double bonds)} \text{ cm}^{-1}. \) The \( \alpha \)-ketol 2 and the diophenol 1 differ significantly in their carbonyl absorption.

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 \( m/e 504 \text{ and } 490, \) which are due to the well established losses of 92 and 106 mass units in the fragmentation of carotenoids.\(^{12-14}\) The peaks at \( m/e 580 \text{ and } 564 \) 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 common reaction in carotenoids,\(^{15,16}\) gives rise to the peak at \( m/e 594 \) and subsequent losses of 92 and 106 mass units account for the peaks at \( m/e 502 \text{ and } 488. \)

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

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

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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 578 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.

An alternative explanation of the mass
spectral data is, however, possible. Although
similar intensity ratios of M - 2 to M peaks have
been observed 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 molecu-
lar 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 492 and 481 μ (diethyl ether) and
spectral fine-structure indicating the presence
of acetylenic bonds (see below). The peracetate
of this presumed tetracene was some-
what 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 absorp-
tion, compared with that reported for alloxa-
thrin, which indicates a close proximity of the
triple bond and the carbonyl function. Moreover,
the bathochromic shift in the visible
light absorption spectrum of "asterinsäure" (3)

_Fig. 2._ Infrared spectra (KBr) of astaxanthin (2) and "asterinsäure" (3).

_Fig. 3._ Mass spectra of astaxanthin (2) and "asterinsäure" (3).

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relative to that of astaxanthin (2) corresponds to that reported for the series alloxanthin (7,8,7',8'-tetrahydro-zeaxanthin), diatoxanthin (7,8-didehydro-zeaxanthin) and zeaxanthin.\textsuperscript{16,17} This can only be explained by assuming a corresponding location of the triple bond(s) in J. Triple bonds in other positions would result in a considerable hypsochromic shift.\textsuperscript{17,18} The relatively marked fine-structure in the electronic spectra of diatoxanthin, alloxanthin,\textsuperscript{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 polyenes 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.\textsuperscript{19}

The experimental procedures used here have been summarized elsewhere.\textsuperscript{29} 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.\textsuperscript{14}

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