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Short Communications

Animal Carotenoids

4.* The Carotenoids of *Asterias rubens* — Asterinsäure

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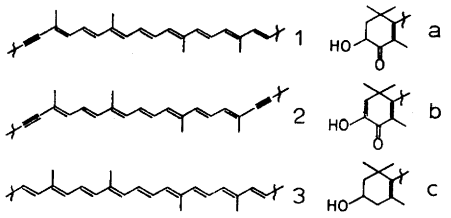
The isolation and structure investigation of asterinsäure, the characteristic carotenoid of the carotenoprotein of the starfish *Asterias rubens*, has been reviewed recently.[1] It was concluded that asterinsäure [2] was 7,8-didehydroastaxanthin (*1a,a*) or a mixture of *1a,a* and 7,8,7′,8′-tetradehydroastaxanthin (*2a,a*).[1] In order to decide between these alternatives a reinvestigation of the carotenoids of *Asterias rubens* has been carried out.



Two isolation procedures were employed. Isolation A involved solvent extraction of the carotenoids and did not lead to pure, crystalline carotenoids because of the large amount of fatty impurities present. Saponification was ultimately resorted to, resulting in autoxidation of the α-ketols to the corresponding diosphenols (*a* → *b*). At this stage the monoacetylenic analogue (*1b,b*) comprised 29 %, the diacetylenic analogue (*2b,b*) 31 %, and a carotenoid heavily contaminated with lipid material and tentatively identified as a alloxanthin (*2c,c*) 34 % of the total carotenoid.

For isolation B the biological material was harvested at a different time. The isolation of the carotenoids was here carried out *via* the crude protein complex, thereby circumventing lipid contamination. In this case the diacetylenic *2a,a* was the only carotenoid isolated. Previous [1,2] and present data indicate that the diacetylenic analogue *2a,a* is the major component of asterinsäure. In isolation A about equal amounts of the mono- (*1a,a*) and diacetylenic (*2a,a*) derivatives were present, whereas in isolation B only the diacetylenic representative *2a,a* was encountered. If the starfish modify the carotenoids present in the diet, the relative amount of acetylenic carotenoids present may be a function of the time elapsed since the last meal.

It has been suggested [3] that only keto-containing carotenoids, frequently with end group *a* such as astaxanthin (*3a,a*), form protein complexes. The results for *Asterias rubens* may be taken to support this hypothesis.

We suggest the misleading trivial name asterinsäure be abandoned and reference be made to the acetylenic carotenoids as 7,8-didehydroastaxanthin (*1a,a*) and 7,8,7′,8′-tetradehydroastaxanthin (*2a,a*).

7,8,7′,8′-Tetradehydroastaxanthin (*2a,a*), bluish crystals from ether (yield *ca.* 3 mg), had m.p. 210°C (uncorr., evacuated tube), previously reported 185°C [2] and 215.5–216°C.[1] Absorption maxima in visible light were at 507 and 541 nm (CS_2), 496 and (526) nm (pyridine), 492 and (522) nm (benzene), and 475 and (496) nm (ether). The spectra in the two former solvents corresponded closely to the curves previously reported.[1] The acetylenic absorption in the IR spectrum (Fig. 1) was relatively stronger than previously recorded,[1] and the PMR spectrum ($CDCl_3$) exhibited in-chain methyl signals of equal

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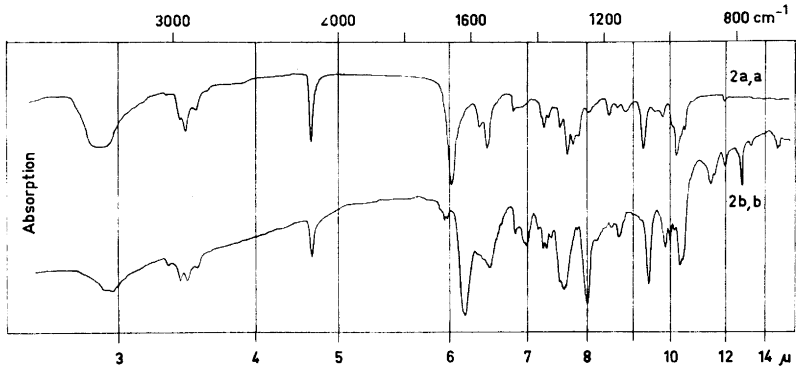

Fig. 1. Infrared spectrum (KBr) of 7,8,7′,8′-tetradehydroastaxanthin (*2a,a*) and the corresponding diosphenol (*2b,b*).

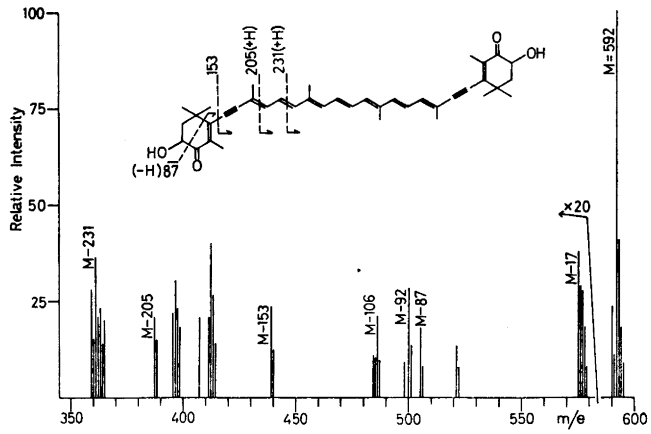

Fig. 2. Mass spectrum of 7,8,7′,8′-tetradehydroastaxanthin (*2a,a*).

intensity at τ 7.98 and 8.02; *cf.* data for alloxanthin (*2c,c*).[4] The mass spectrum (Fig. 2) had its molecular ion peak at *m/e* 592 and characteristic M−92 and M−106 peaks.[5,6] The allocation of the triple bonds to 7,8,7′,8′-positions follows from visible light and infrared absorption data [1] and is supported by the PMR data. In agreement with this assignment no species corresponding to the *m/e* 152 and *m/e* 137 ions observed by Baldas *et al.*[7] in the case of astacene (*3b,b*) were observed and M−90 ions characteristic of carotenoids with centrally located triple bonds [7,8] were absent. Peaks at M−153, M−205, and M−231 are compatible with in-chain fragmentations, Fig. 2. It should be pointed out that the presence of an M−106 peak in *2a,a* is not in accordance with the currently accepted mechanism [9,10] for the formation of the M−106 ion, although it may be explained by the mechanism earlier suggested.[6] M−106 peaks were not observed for the diosphenols *1b,b* and *2b,b*.

In the iodine catalyzed equilibrium mixture [11] the *trans* isomer comprised 63 % and a neo A (kieselgur paper) isomer (λ_{max} 468 nm in ether) 38 % of the total carotenoid. The derivatives of *1* and *2* studied here all showed a marked tendency to undergo *cis* isomerization.

The diacetate of *2a,a* prepared by standard acetylation [13] had molecular ion at *m/e* 576.

The diosphenol *2b,b*, obtained on alkali treatment,[12] showed less spectral fine-

structure in visible light, and the acetylenic absorption in the IR spectrum was less pronounced; characteristic ν_{max} (KBr, Fig. 1) 2160, 1620, 1540, 1250, 1065, 970 cm^{-1}. Mass spectrum:[13] *m/e* 588 (M), M−92.

7,8-Didehydroastaxanthin (*1a,a*) was not obtained in the pure state. *1a,a* exhibited absorption in visible light at 473 nm (ether) with less spectral fine-structure than *2a,a*.

The corresponding diosphenol (*1b,b*) had spectral fine-structure in visible light (CS_2) intermediate between astacene (*3b,b*) and *2b,b*. Except for less intense acetylenic absorption at 2160 cm^{-1}; the IR spectrum (KBr) was very similar to that of *2b,b*. Mass spectrum:[13] *m/e* 590 (M), M−92, *m/e* 152, and 137 ions [7] were observed.

Alloxanthin (*2c,c*), heavily contaminated with lipids, was tentatively identified from the mass spectrum (*m/e* 564 (M), M−92), electronic spectrum (abs.max. 452 nm with better spectral fine-structure than β-carotene) and strong tendency to *cis*-isomerize to isomers with intense *cis* peak [14] and from its approximate polarity.

Experimental. For isolation A *Asterias rubens* (12 kg live weight, collected at Espegrend Biological Station, University of Bergen, in March 1969) was used. The extractable carotenoids amounted to 51 mg. In isolation B a similar portion harvested at the same locality on February 1970 was used. In both cases only the back skins were extracted. Isolation A involved extraction by acetone, transfer of the pigments to ether, standard saponification,[13] separation into neutral and acidic carotenoids, subsequent precipitation of non-carotenoid material from cold acetone and chromatography of the neutral carotenoids on alumina and the acidic ones repeatedly on cellulose columns and subsequent precipitation. For isolation B the initial steps of the procedure of von Euler and Hellström [3] was used. The crude protein complex was extracted with ether and the carotenoids chromatographed repeatedly on cellulose columns. Materials, methods and instruments used are specified elsewhere.[12,15] Mass spectra were recorded on an AEI MS 902 instrument at 70 eV at lowest possible ion source temperature. The α-ketols and diosphenols were eluted from the cellulose columns with mixtures of ether and petroleum ether; the monoacetylenes being less strongly adsorbed than the diacetylenes. Column fractions were examined by circular chromatography on kieselgur paper [16] using the system 10 % acetone in petroleum ether. Again the diacetylenic analogues were slightly more strongly adsorbed than the monoacetylenic ones and the diosphenols more retained than the α-ketols.

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