Animal Carotenoids. 16*. Tunaxanthin

HARALD RØNNEBERG,[a] GUNNER BORCH,[b] SYNNØVE LIAAEN-JENSEN,[a] HISAKO MATSUTAKA [c] and TAKAO MATSUNO [c]

[a] Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway, [b] Chemistry Department A, Technical University of Denmark, DK-2800 Lyngby, Denmark and [c] Kyoto College of Pharmacy, Misasagi Yamashina, Kyoto, Japan

Tunaxanthin, first isolated by Hirao *et al.*[1] and partly characterized by Crozier and Wilkie,[2] now appears to be a common carotenoid of various marine fishes.[3–12].

On the basis of its electronic spectrum, partition ratio, negative epoxide test and formation of a presumed dimethyl ether under conditions for allylic methylation structure *1* has been assigned to tunaxanthin.[2] Further spectroscopic and chemical evidence in support of structure *1* has not been reported and the chirality of tunaxanthin has remained unknown.

Weedon [13] some years ago gave a preliminary report on the total synthesis of optically inactive *1a* (3,6-*cis*, 3′,6′-*cis*) and optically inactive *1b* (3,6-*trans*, 3′,6′-*trans*) and stated that a concentrate of tunaxanthin by ^{1}H NMR compared favourably with *1b*.

More recently Bingham *et al.*[14] have assigned structure *1c* (3,6-*cis*, 3′,6′-*trans*) and *1a*, respectively, to chiriquixanthin A and B obtained from a frog. The relation between tunaxanthin and the chiriquixanthins has, however, not been established.

In the present work tunaxanthin *ex Amanses modestus*, *ex Thunnus thynnus* and *ex Seriola quinqueradita* has been subjected to a modern examination. Chromatographically homogeneous tunaxanthin exhibited λ_{max} (acetone) at 417, 441 and 471 nm, % III/II [15] = 92, consistent with the aliphatic nonaene chromophore and was chromatographically (R_F = 0.45, SiO_2 plate, 50 % acetone in hexane) slightly less strongly adsorbed than lutein (*2*). Mass spectral data confirmed the $C_{40}H_{54}(OH)_2$ structure: *m/e* 568, 26.7 % (M); M − 18 and M − 18 − 18. The allylic character of the two hydroxy groups

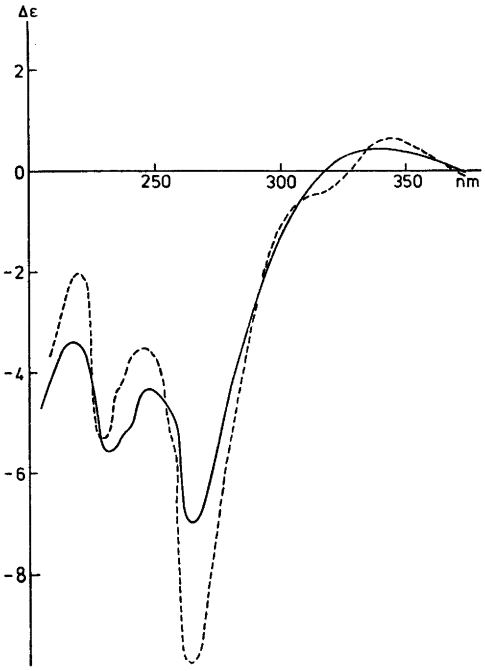
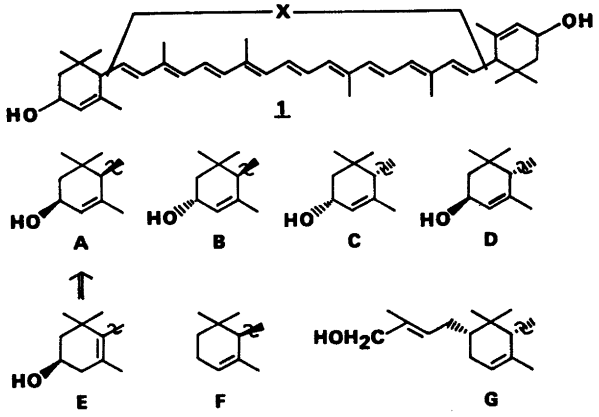


Fig. 1. CD spectra in EPA solution (diethyl ether – isopentane – ethanol 5:5:2) of natural tunaxanthin (*1d*, ———) and tunaxanthin 3,3′-dimethyl ether (- - -).

* No. 15. *Biochim. Biophys. Acta. In press.*



1a A–x–A
1b B–x–B
1c A–x–B
1d C–x–D
2 E–x–B
3 F–x–F
4 G–x–G
5 E–x–E

Table 1. ^{1}H NMR data (δ) for tunaxanthin and related carotenoids.

Carotenoid	Me-16,17	Me-18	Me-19,19′	Me-20,20′	Me-16′,17′	Me-18′
Tunaxanthin [a]	0.94;0.852	1.63	1.906	1.959	0.999;0.852	1.64
Lutein (2)[25]				1.96	0.99; 0.84	1.62
Lutein (2)[26]				1.95	1.00; 0.83	1.63
Chiriquixanthin A (*1c*) [14]	0.94;0.84	1.63	1.90	1.95	0.99; 0.84	1.63
Chiriquixanthin B (*1a*) [14]	0.94;0.84	1.63	1.90	1.95	0.94; 0.84	1.63

[a] H-3,3′ 4.1-4.4; H—4,4′ 5.2—5.7; other olefinic H 5.9—6.9.

was proved by methylation with acidified methanol [16] in a parallel experiment with lutein (*2*). Tunaxanthin provided an intermediary monomethyl ether and a still less strongly adsorbed dimethyl ether exhibiting unchanged electronic spectrum and mass spectral properties with *m/e* 596, 3.8 %, M − 32 and M − 32 − 32.

^{1}H NMR data for tunaxanthin were consistent with the general structure *1*, but revealed different chemical shifts of the two end groups, compatible with one 3,6-*cis* and one 3,6-*trans* ε-ring as in chiriquixanthin A [1] (see Table 1).

It is now well known that the CD spectra of carotenoids containing substituted ε-rings are determined nearly exclusively by the chirality at C-6,6′.[17–20] The CD spectra of tunaxanthin (Fig. 1) with negative Cotton effect below 320 nm strongly indicated 6*S*,6′*S*-chirality (as in end groups C and D), in comparison with the chiroptical properties of natural ε,ε-carotene (*3*),[21] decaprenoxanthin (*4*) [18] and the chiriquixanthins (*1a* and *1c*). This was confirmed by the CD properties (Fig. 1) of the tunaxanthin dimethyl ether which corresponded to those of natural tunaxanthin. The methylation reaction is presumed to occur largely with racemization by an S_N1 reaction, recently confirmed by HPLC separation of the two epimeric lutein 3′-methyl ethers prepared in the same way.[22] Were the chirality of the C-6,6′ centers opposite, hardly any Cotton effect would be expected for either tunaxanthin dimethyl ether or tunaxanthin.

Accepting 6*S*,6′*S* chirality for tunaxanthin from its chiroptical properties and one 3,6-*cis* and one 3′,6′-*trans* end from the ^{1}H NMR evidence (Table 1) these data lead to structure *1d* for tunaxanthin ≡3*R*,6*S*,3′*S*,6′*S*-ε,ε-carotene-3,3′-diol, enantiomeric with chiriquixanthin A (*1c*).

In the frog the chiriquixanthins A and B could possibly be derived from dietary zeaxanthin (*5*) and lutein (*2*),[23] respectively, by stereospecific isomerization of the Δ5 double bond in end group *E* to Δ4 of end group *A*.

Lutein (*2*) is a less attractive precursor of tunaxanthin (*1d*) since an epimerization at C-6′ would be required, and the chirality of tunaxanthin (*1b*) in biochemical context seems rather obscure. The natural occurrence of both *1a*, *1c* and *1d* suggests more stereochemical variation in naturally occurring carotenoids than previously assumed. In particular it calls for attention in assuming identical chirality in different sources.

1. Hirao, S., Yamada, J. and Kikuchi, R. *Bull. Tokai Reg. Fish. Res. Lab. 16* (1957) 53.
2. Crozier, G. F. and Wilkie, D. W. *Comp. Biochem. Physiol. 18* (1966) 801.
3. Crozier, G. F. *Comp. Biochem. Physiol. 23* (1967) 179.
4. Katayama, T., Hirata, K., Yokoyama, H. and Chichester, C. O. *Bull. Jpn. Soc. Sci. Fish. 36* (1970) 709.
5. Katayama, T., Shintani, K. and Chichester, C. O. *Comp. Biochem. Physiol. B 44* (1973) 253.
6. Katayama, T., Miyaharo, T., Tanaka, Y., Sameshima, M., Simpson, K. L. and Chichester, C. O. *Bull. Jpn. Soc. Sci. Fish. 40* (1974) 97.
7. Matsuno, T., Nagata, S., Sato, Y. and Watanabe, T. *Bull. Jpn. Soc. Sci. Fish. 40* (1974) 619.
8. Matsuno, T. and Katsuyama, M. *Bull. Jpn. Soc. Sci. Fish. 41* (1975) 675.
9. Czezuga, B. *Hydrobiologia 46* (1975) 223.
10. Matsuno, T. and Katsuyama, M. *Bull. Jpn. Soc. Sci. Fish. 42* (1976) 761.
11. Matsuno, T. and Katsuyama, M. *Bull. Jpn. Soc. Sci. Fish. 42* (1976) 651.
12. Matsuno, T., Katsuyama, M. and Takashi, A. *Bull. Jpn. Soc. Sci. Fish. 42* (1976) 765.
13. Weedon, B. C. L. *Pure Appl. Chem. 47* (1976) 161.
14. Bingham, A., Mosher, H. F. and Andrewes, A. G. *Chem. Commun.* (1977) 96.
15. Ke, B., Imsgaard, F., Kjøsen, H. and Liaaen-Jensen, S. *Biochim. Biophys. Acta 210* (1970) 139.
16. Liaaen-Jensen, S. and Hertzberg, S. *Acta Chem. Scand. 20* (1966) 1703.
17. Andrewes, A. G., Borch, G. and Liaaen-Jensen, S. *Acta Chem. Scand. B 30* (1976) 214.

18. Andrewes, A. G., Liaaen-Jensen, S. and Weeks, O. B. *Acta Chem. Scand. B 29* (1975) 884.
19. Hertzberg, S., Borch, G. and Liaaen-Jensen, S. *Acta Chem. Scand. 32* (1978). *In press.*
20. Bodea, C., Andrewes, A. G., Borch, G. and Liaaen-Jensen, S. *Phytochemistry. In press.*
21. Buchecker, R. and Eugster, C. H. *Helv. Chim. Acta 54* (1971) 327.
22. Fiksdahl, A., Mortensen, T. and Liaaen-Jensen, S. *J. Chromatogr. In press.*
23. Buchecker, R. and Eugster, C. H. *Helv. Chim. Acta 56* (1973) 1124.
24. Andrewes, A. G., Borch, G. and Liaaen-Jensen, S. *Acta Chem. Scand. B 28* (1974) 139.
25. Buchecker, R., Hamm, P. and Eugster, C. H. *Helv. Chim. Acta 57* (1974) 631.
26. Nitsche, H. *Arch. Mikrobiol. 95* (1974) 79.

Received June 9, 1978.

Dry Ozonation of 3β, 28-Diacetoxylupane. A Comment on the Structure of a Pentacyclic Triterpenoid Lactone from *Dillenia indica* (Linn.)

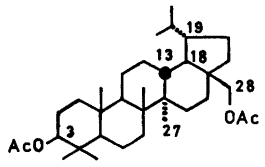
ELIAS SUOKAS and TAPIO HASE

Department of Chemistry, Helsinki University of Technology, SF-02150 Espoo 15, Finland

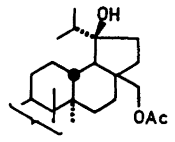
Recently dry ozonation – *i.e.* the ozonation of a substrate adsorbed on a carrier (*e.g.* silica gel) without solvent – has been found to be a valuable tool for functionalisation at an unactivated carbon.[1] This oxygenation is often remarkably regioselective and stereospecific with retention of configuration. Usually oxidation at a tertiary carbon to give an alcohol is preferred but attack at secondary carbons may also occur.

Triterpenes are an interesting group for testing this functionalisation method due to their rigid stereostructure, to the environmental diversity of carbon centres, and to the opportunity of selective functionalisation within the fully saturated carbon skeleton – a tedious task by other means. In this field the dry ozonation of a friedelane hydrocarbon was very recently reported[1b] to yield compounds resulting from oxidation at secondary carbons. A higher selectivity is shown[1] by substrates containing polar groups, which regulate the orientation of the molecule on the adsorbent and thus direct the spatial attack of ozone.

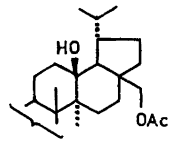
We now report that the dry ozonisation of 3β,28-diacetoxylupane (*1*) on silica gel yields only one product (conversion *ca.* 10 %). Its IR spectrum showed the presence of a hydroxyl group and the ^{1}H NMR spectrum revealed its tertiary nature. The protons at C-28 appeared at exceptionally low field in the ^{1}H NMR spectrum indicating that the new hydroxyl is located in the neighbourhood of C-28.



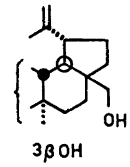
1



2



3



4

The suitable tertiary positions on the β-face are at C-13 and C-19. The physical constants of the ozonation product are in rather good agreement with the known[2] 19β-hydroxy compound *2* and differs clearly from those reported[3] for the 13β-hydroxy compound *3*. There was, however, some doubt about the correctness of the reported[3] structure of *3* and therefore we have prepared both of these hydroxy diacetates *2* and *3* for identification. The 19β-hydroxy compound *2* is available[2] from betulin (*4*) and the 13β-hydroxy compound *3* was prepared from the known[4] ether *5 via* $NaIO_4-RuO_2$ oxidation to the lactone *6*, $LiAlH_4$ reduction and reacetylation to the hydroxy diacetate *3*. The hydroxy diacetate from the dry ozonation of 3β,28-diacetoxylupane (*1*) was found to be identical (m.p., mixed m.p., $[\alpha]_D$, TLC, IR, ^{1}H NMR, *m/e*) with the 19β-hydroxy derivative *2*.