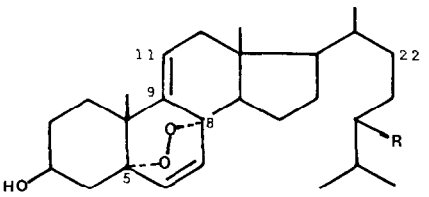
Tetrahedron Letters, Vol. 22, No. 15, pp 1391 - 1392 ,1981 0040-4039/81/151391-02$02.00/0
Printed in Great Britain

OCCURRENCE OF 9(11)-UNSATURATED STEROL PEROXIDES IN TUNICATES.

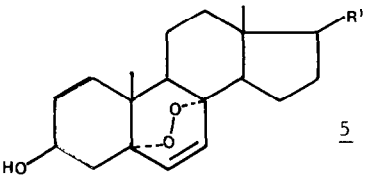
Michèle GUYOT * and Marguerite DURGEAT
Laboratoire de Chimie du Muséum National d'Histoire Naturelle, 63 rue Buffon, 75005 - PARIS.

Abstract - New natural 9(11)-unsaturated sterol peroxides 1, 2, 3, 4 have been isolated from marine Tunicates (Urochordates). Structure elucidation was based on spectral data and confirmed by synthesis.

In the course of our search of biologically interesting molecules from Tunicates, four new naturally occurring peroxides 1 to 4 were isolated among which 3 had previously been synthetized (1) as starting material for a synthesis of cortisone.



1 R : H
2 R : $=CH_2$
3 R : CH_3 Δ^{22}
4 R : CH_2-CH_3



5

Two solitary Tunicates collected on French coasts : *Phallusia mamillata* in Etang de Thau and *Ciona intestinalis* in Corse, were lyophilized, grounded and extracted in dark with dichloromethane. Crude extracts of each Tunicate were submitted to repeated separations on silica gel columns and plates. Beside a crystalline mixture (0.3 % dry weight) of 5α,8α-sterol peroxides 5, obtained earlier from sponges by Djerassi (2) and Minale (3), we isolated another fraction (0.06 % dry weight) also exhibiting the characteristic features of sterol peroxides.

It was found by MS that this fraction was a mixture from which preparative TLC on reversed-phase layers (4) enabled us to obtain from *P.mamillata* four compounds 1 to 4 and from *C.intestinalis* the only compound 1, in reasonable amounts permitting spectral analysis.

By high resolution mass measurements their respective molecular formulae were established:

1 $C_{27}H_{42}O_3$ (1.7 mg), M^+ measured 414.3142 ; calculated 414.3134 ,
2 $C_{28}H_{42}O_3$ (2.1 mg), M^+ measured 426.3131 ; calculated 426.3134 ,
3 $C_{28}H_{42}O_3$ (1.1 mg), M^+ measured 426.3139 ; calculated 426.3134 ,
4 $C_{29}H_{46}O_3$ (0.8 mg), M^+ measured 442.3449 ; calculated 442.3446 .

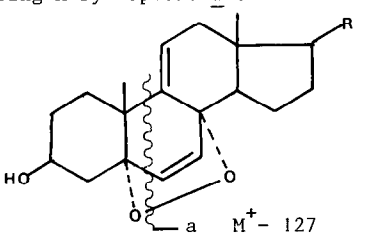
The NMR of the four compounds presented signals for olefinic protons as two doublets, J = 8 Hz, at δ 6.25 and 6.60 ppm. The deshielding of these values, compared with δ 6.20 and 6.55 for 5 (2,3) and a quartet at δ 5.41 ppm strongly suggested the presence of an additional double bond in the vicinity to the C-5, C-8 system. This double bond could be located at 9(11) or 14(15). Position 9(11) seemed the biogenetically more favoured one and was in accordance with NMR and MS spectral data. Hence, $\Delta^{9,11}$-cholesterol peroxide and $\Delta^{9,11}$-ergosterol peroxi-

des were synthesized following (1) ; NMR and MS parameters appeared respectively identical to those of natural 1 and 3. In NMR, caracteristic common signals were found at δ (ppm) 6.60 (d J=8.5) , 6.25 (d J=8.5), H-6, H-7 ; 5.41 (q J=5.5 ; 2.5) H-11 ; 4.02 (m) H-3 ; 2.32 (q J= 17 ; 5.5) H-12β ; 1.09 (s) Me-19 ; 0.72 (s) Me-18.

In mass spectra, peaks corresponding to M^+, $M^+ - O_2$, M^+ - SC for 1 and 4 and M^+ - SC-2H for 2 and 3, m/e 251 M^+ - SC- O_2-H_2O and m/e 209, loss of ring from m/e 251, were found.

The nature of the side chain of 2 was deduced from the following spectroscopic facts : in NMR signals at 4.72 (m) C-28 H and 0.98 (d) C-26,27 Me (2) and in MS : base peak at m/e 299.2647, $C_{19}H_{23}O_3$, corresponding to M^+ - SC and ion m/e 383 $M^+ - C_3H_7$.

In addition, during examination of the mass spectra of these compounds, our attention was focused on the appearance of important ions at m/e 287 in 1 and m/e 315 in 4, corresponding to M^+ - 127. An analogous ion at m/e 299 in 2 and 3 could be interpreted as the loss of the side chain with 2H transfert. High resolution mass measurements allowed us to attribute the formula $C_{20}H_{31}O$ (6) to the ion at m/e 287 in 1, and to resolve ion m/e 299 in 2 into a doublet in which the first ion corresponds to the loss of the side chain (*vide supra*) and the second one to the formula $C_{21}H_{31}O$ (7). These formulae allowed us to interpret this common fragmentation as the loss of ring A by rupture a .



a M^+ - 127

The products described are probably generated by photosensitized oxygenation of the corresponding trienes since pigments are present in animals. To prevent such an oxidation during work-up, extraction was effected in darkness with the same results ; thus allowed us to deduce that these products are true metabolites of Tunicates.

The biological activity of these compounds presently under study will be described later.

Acknowledgment :

We wish to thank D.Davoust for NMR spectra, J.P.Brouard for the mass spectra, J.Mercier for high resolution mass spectra and F.Monniot who collected *Ciona intestinalis*.

References :

(1) BLADON P., CLAYTON R.B., GREENHALGH C.W., HENBEST H.B., JONES E.R.H., LOVELL B., SILVERSTONE G., WOOD G.W. and WOODS G.F., J.chem.Soc., 1952, p.4883.

(2) SHEIKH Y.M. and DJERASSI C., Tetrahedron, 1974, *30*, 4095.

(3) MALORNI A., MINALE L. and RICCIO R., Nouv.J.Chim., 1978, *2*, 351.

(4) The Rf of the compounds are 2 > 3 > 1 > 4 in MeOH-H_2O, 95/5 (2x),Whatman plates KC 18.

(5) mp 169-172°C, $[\alpha]_D^{20}$ = + 97.99° (c 1.5, $CHCl_3$)

(6) Measured 287.237 ; calculated 287.2375.

(7) Measured 299.238 ; calculated 299.2375.

(Received in France 28 November 1980)