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CHOLEST-4-EN-4,16β,18,22R-TETROL-3-ONE 16,18-DIACETATE A NOVEL POLYHYDROXYLATED STEROID FROM THE HYDROID *EUDENDRIUM* SP.

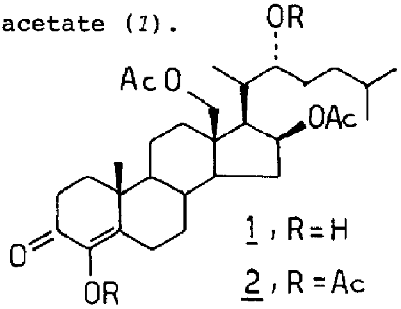
G. Cimino,S. De Rosa,S. De Stefano and G. Sodano

Istituto di Chimica di Molecole di Interesse Biologico del CNR,
Via Toiano n. 2,Arco Felice,Naples,Italy

ABSTRACT: The title compound has been isolated from the hydroid *Eudendrium* *sp. (Cnidaria,Hydrozoa,Hydroidea) and its structure elucidated by spectral means.*

There are three species of the hydroid *Eudendrium (rameum,racemosum,ramosum)* closely living in the bay of Naples,which are prey of several nudibranchs (e.g. *Hervia peregrina,Flabellina affinis,Coryphella lineata*) and on which the same nudibranchs deposit their eggs.In view of the high potential of these associations[2] for the study of some basic biological problems such as the induction of metamorphosis in veligers, chemorecognition and chemotaxis,we decided to investigate the steroid content of predators and preys.

We have found in the hydroids and in the nudibranchs the same pathway of polyhydroxylated steroids and we describe here the structure of the major component, cholest-4-en-4,16β,18,22R-tetrol-3-one 16,18-diacetate (*1*).



1 , R=H

2 , R=Ac

The diethyl ether soluble fraction of the acetonic extracts of *Eudendrium* was chromatographed on silica gel ($CHCl_3$-CH_3OH,98:2) and the fractions containing *1* were further purified by HPLC (Bondapak C_{18};CH_3OH-H_2O,65:35).

1, $C_{31}H_{48}O_7$,0.018% dry weight,was recovered as an amorphous solid,M^+ 532,$[\alpha]_D^{25}$ +55° (c,0.4;$CHCl_3$);λ_{max} (CH_3OH) 277 nm,ε 9.900; λ_{max}(CH_3OH-NaOH) 314 nm;ν_{max} 3600,1730,1665,1635 and 1230 cm^{-1}.

A substituted cholestane nucleus was inferred from the p.m.r. spectrum ($CDCl_3$) showing the following signals:δ 0.90 (6H,d;J 7Hz;isopropyl Me's),1.09 (3H,d;J 7Hz; 21-Me),1.20 (3H,s;19-Me),2.05 (3H,s;CH_3-CO-),2.12 (3H,s;CH_3-CO-),3.45 (1H,m;$C\underline{H}OH$), 4.38 (2H,ABq;J 12Hz;$C\underline{H}_2OAc$),5.10 (1H,m;$C\underline{H}OAc$).

The absence of a methyl singlet assignable to the 18-Me coupled with the presence of the ABq δ 4.38 (δ 63.1 in the c.m.r. spectrum) clearly indicate the presence of an acetoxy group at C-18,a functionalization recently found in a pregnane derivative from marine source[3].

The presence of an OH group at C-22 was deduced from the significative M^+-10 m.u. peak in the mass spectrum of *1* (20-22 cleavage + 1H)[4] and from the chemica shift[5] of the 21-Me in the c.m.r. spectrum (δ 12.2). Furthermore the chemical shift of the C-23 at δ 28.0 in the c.m.r. spectrum of *1*, shifted to δ 23.9 in th c.m.r. spectrum of the tetraacetate (*2*), obtained by acetic anhydride-pyridine treatment of *1*, was indicative[5] for a 22R configuration.

The diosphenol moiety in the ring A, suggested by the u.v. data, was confirmed by comparison of the c.m.r. spectra of *1* and *2* with that of 4-hydroxy-cholest-4-en-3-one (*3*)[6] and of its acetyl derivative (*4*). Besides the close similarity of the chemical shifts for the relevant carbons between the natural and the model compounds[7], the comparison of the c.m.r. spectra allowed the assignment of the carbons of the rings A and B; moreover the chemical shift of the C-11 (δ 20.6) in the c.m.r. spectrum of *1* excluded the functionalization of the ring C, the CHOAc group being confined in the ring D.

The chemical shifts of C-14 and C-17 (δ 53.7 and 57.1 or *viceversa*) allow the attachment of the CHOAc at either C-15 or C-16. However the chemical shift of C-20 (δ 37.7) significantly lower than that of model compounds ($\delta\sim$42)[5] can be justified only by an additional γ-gauche effect coming from a 16β acetoxy group. Therefore the assignment of the chemical shift for C-14 and C-17 should be 53.7 and 57.1 respectively.

Work is in progress for the structural elucidation of the remaining component of the steroidal mixture.

REFERENCES AND NOTES

1. This work is a part of the "Progetto Finalizzato per l'Oceanografia e i Fond Marini", CNR, Roma.

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7. *1*, δ ($CDCl_3$): C-2, 31.8; C-3, 193.4; C-4, 141.4; C-5, 138.9; C-6, 22.7; C-10, 35.8.
   2, δ ($CDCl_3$): C-2, 33.3; C-3, 190.3; C-4, 154.8; C-5, 139.0; C-6, 24.9; C-10, 39.0.
   3, δ ($CDCl_3$): C-2, 31.1; C-3, 193.5; C-4, 141.2; C-5, 140.3; C-6, 23.1; C-10, 35.3.
   4, δ ($CDCl_3$): C-2, 33.5; C-3, 190.6; C-4, 156.1; C-5, 139.2; C-6, 24.2; C-10, 39.2.

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