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HIPPURIN-1, AN UNUSUAL STEROID FROM THE GORGONIAN *ISIS HIPPURIS*

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Chemical investigation of gorgonians has been intense and a large number of terpenoid and acetate derived metabolites have been discovered[1]. Several novel sterols, of which the C_{30} sterol gorgosterol (1)[2] is the best known, have been reported from gorgonians. We now describe the isolation and structural elucidation of hippurin-1 (2) from *Isis hippuris*, a common gorgonian found on the Great Barrier Reef.

Percolation of the milled freeze-dried organism with cold dichloromethane gave a 1.7% extract which was partitioned between petroleum ether and 95% methanol. Evaporation of the methanol layer gave a syrup representing half of the original extract. Chromatography of this mixture on silica gel using a dichloromethane-ethyl acetate gradient gave, in order of elution, a sterol related to (2) for which we propose the name hippurin-2 (3%), gorgosterol (1) (1.3%), hippurin-1 (2) (20%) and an orange pigment (0.6%), identified as peridinin (3)[3].

Hippurin-1 crystallised from diethyl ether-petroleum ether as small rosettes[4]. The formula $C_{30}H_{48}O_7$ was established by elemental analysis and high resolution mass spectral data on the first fragment peak at 505 a.m.u. (M^+ - 15).

The ^1H n.m.r. spectrum of (2) indicated the presence of the grouping $-CH_2-CH(OAc)-CH(OH)-CH_2$ (δ2.04, 3H,s; δ4.97, 1H,m, $W_{h/2}$ 16 Hz; coupled to a signal at δ4.03, 1H,m, $W_{h/2}$ 7Hz). The data suggested that the acetoxyl group was equatorial and the hydroxyl group was axial. Two further -CH-O- signals were centred at δ4.25.

Acetylation and oxidation of (2) proceeded sequentially and both mono- and di-oxidation products (4) and (5), and both acetylation products (6) and (7), were obtained which established the presence of two secondary hydroxyl groups. Acetylation followed by oxidation or oxidation followed by acetylation produced the same product (8) ($C_{32}H_{48}O_8$). Therefore one proton of the two centred at δ4.25 in the ^1H n.m.r. spectrum of (2) must be due to a -CH-OH group and the other due to a secondary ether function.

The ^{13}C n.m.r. of (2) showed all thirty carbon atoms. A low field signal of the acetate carbonyl appeared at δ170.1 and a singlet at δ115.1 (O-$\overset{|}{\underset{|}{C}}$-O) was the only other resonance below δ100. Therefore (2) was hexacyclic. The presence of six methyl groups suggested the possibility of a C_{28} sterol nucleus with the additional methyl group at C24. The mass spectra of (2) and derivatives (4) - (8) all showed a base peak at 129 a.m.u. ($C_7H_{13}O_2$) which was independent of acetylation or oxidation and therefore probably arose by a C20-C22 cleavage of the sterol side

chain with possible partial structures $(CH_3)_2C(25)$-O and $-CH(CH_3)-CH_2-\overset{O}{\overset{\|}{C}}(22)$-O and therefore the two rings extra to the usual tetracyclic sterol nucleus could be rationalised as cyclic ethers incorporated into the sterol side chain. This was supported by ^{13}C n.m.r. evidence which showed that, in addition to the signal at δ115.1, there were six further signals due to carbon atoms bonded to an oxygen. Two singlets could be ascribed to a tertiary alcohol (C20) and tertiary ether (C25) (δ84.5 and 79.3) and four doublets could be rationalised as one acetoxyl, two hydroxyl and one secondary ether functions respectively.

The positions of one equatorial acetoxyl group and two axial secondary hydroxyl functions remained to be assigned. If the sterol nucleus was accepted the spectral requirements for a $-CH_2-CH(OAc)-CH(OH)-CH_2-$ grouping demanded that the 2 and 3 positions of a sterol nucleus were oxygenated and the possible partial structure (9) emerged where the 'extra' secondary hydroxyl group must be in ring B or C (i.e. evidence on (5)) and must be axial.

A single crystal X-ray analysis of the monoacetate (6) secured the structure and relative stereochemistry of hippurin-1 as (2).

The compound (6) separated from methanol as monoclinic needles, space group $P2_1$, with $\underline{a}$ = 18.556 ± 0.004, $\underline{b}$ = 7.445 ± 0.002, $\underline{c}$ = 11.890 ± 0.003 Å, β = 106.04° ± 0.03, α = γ = 90° with two molecules in the unit cell. Solution was achieved by direct methods from 4067 measured reflections of which 2158 were taken to be observed. The phase problem was solved with a multisolution method and tangent formula refinement using the programme MULTAN/1/. The starting set of phases was chosen by ourselves because the set chosen automatically by this programme failed.

Atomic co-ordinates were refined by block-diagonal least squares and after 23 L.S. cycles an R-value of 15.80% was found. The stereoprojection, representing the enantiomer with the true absolute configuration, is shown in Figure 1.

Monoacetylation of hippurin-1 (2) resulted in the shift of a $O-\overset{|}{C}-H$ proton in the ^1H n.m.r. spectrum from δ4.03 to δ5.33. The $W_{h/2}$ of 7Hz for this proton demanded an equatorial configuration and therefore the -OH group from which the acetate was derived must be axial. This necessitated an α-C3 hydroxyl group and an α-C2 acetoxyl group in hippurin-1 which could be written as (2).

O.R.D. studies on the monoketone (4) and the diketone (5) established the absolute configuration shown, the normal absolute configuration found in sterols.

ACKNOWLEDGEMENTS

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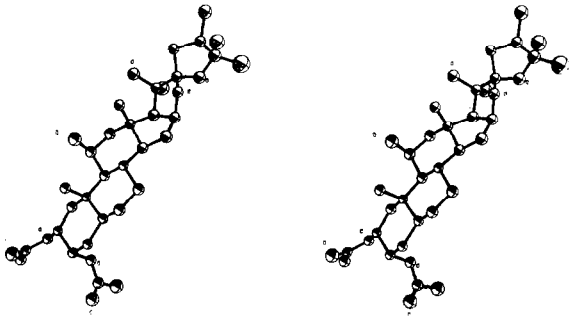
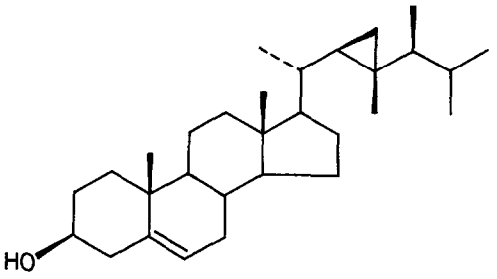
4. M.p. 183-185°; $[\alpha]_D$ + 36.2°(c=1, $CHCl_3$); i.r. (KBr disk) 1735 cm^{-1}.

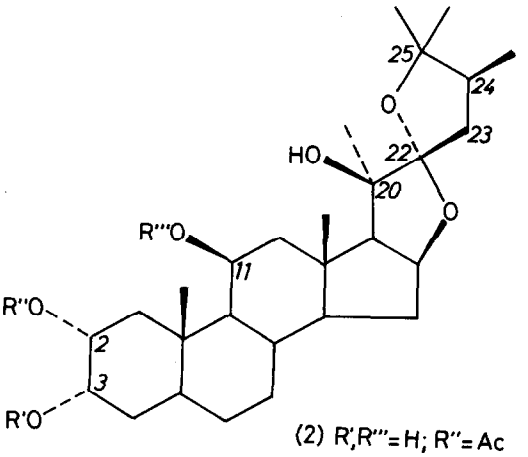
FIGURE 1

Stereoprojection of hippurin-1 monoacetate.





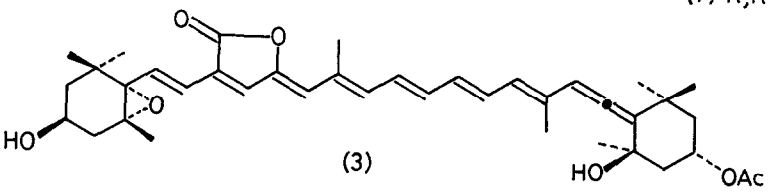
(1)



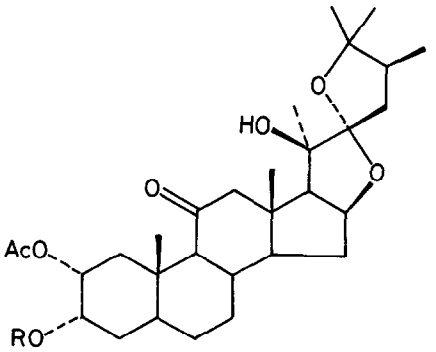
(2) R',R'''=H; R''=Ac

(6) R',R''=Ac;R'''=H

(7) R',R'',R'''=Ac

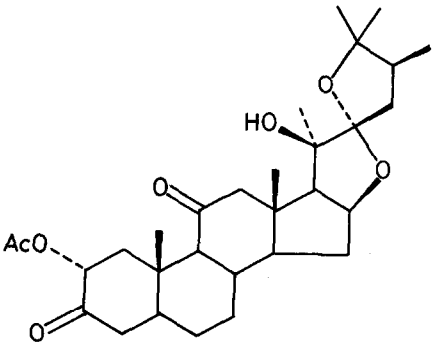


(3)

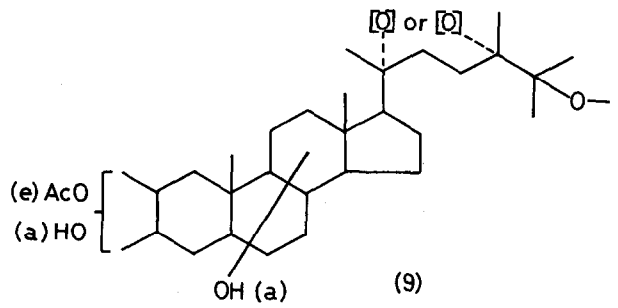


(4) R=H

(8) R=Ac



(5)



(9)