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# OCCURRENCE OF 19-NOR CHOLESTEROL AND HOMOLOGS IN MARINE ANIMALS

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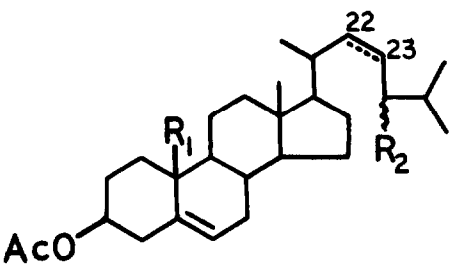
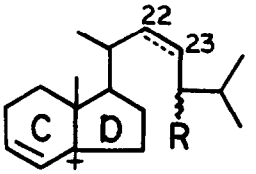
Our current interest[1] in novel marine sterols representing potential "missing links" in certain unexpected biosynthetic pathways (e.g., C-22 and C-23 alkylation) prompted us to examine the gorgonian Pseudoplexaura porosa[2] - a rich source of the biogenetically intriguing marine sterol gorgosterol.[3] Utilizing our recently described separation scheme[4] we encountered over 40 sterols, including members of the important 19-nor steroid class which form the subject of this paper.

Preliminary separation of the total sterol acetates was effected by column chromatography on neutral alumina (act. I) impregnated with 30% $AgNO_3$. The slowly moving constituents eluted with 10:2 hexane-benzene were subjected to GC- high resolution MS analysis using a Hewlett Packard 7610A gas chromatograph equipped with a 10' x 2 mm "U" shaped column [3% Poly S-179 on gas chrom Q or 3% OV-17 on gas chrom Q (column temp 260°)] and interfaced with a Varian Mat 711 double focussing mass spectrometer (equipped with a Watson-Biemann dual stage separator, an all glass inlet system and a PDP-11/45 computer for data acquisition) operating at a resolution of 5000, thus providing an "element map".[5] Three of the peaks with retention times 0.92, 1.15 and 1.35 (1.0 = cholesterol acetate) correspond to pure components (approximate ratio of 2:3:1), which according to their mass spectra are all members of a homologous series.

The fastest moving sterol acetate displayed a simple mass spectrum with significant (>15% relat. int.) peaks in the high mass range occurring only at m/e 354 (base peak, M-HOAc), 349, 247, 241 and 199. The intense loss of acetic acid and the absence of a molecular ion peak is typical of $\Delta^5$-3β-acetoxy steroids such as cholesterol acetate (IV)[6] (M - HOAc = 368), whose other diagnostic high mass range peaks are found at m/e 353 (M - HOAc + $CH_3$), 260 (unknown origin), 255 (M - HOAc + side chain), 247 (fission[6] of allylic 7, 8 and 9, 10 bonds-H) and 213 (M - HOAc +




I, $R_1$=Me;$R_2$=H
II, $R_1$=$R_2$=H
III, $R_1$=H;$R_2$=Me
IV, $R_1$=Me;$R_2$=Et;$\Delta^{22}$
V, $R_1$=$H_1$;$R_2$=Et;$\Delta^{22}$




VI, m/e 247;R=H
VII, m/e 261;R=Me
VIII, m/e 273;R=Et;$\Delta^{22}$

ring D fission[7]). The fact that the new sterol acetate was more mobile than cholesterol acetate (I) and that all relevant high mass range peaks (m/e 260 or any counterpart were absent) except for m/e 247 were shifted downwards by 14 mass units strongly suggested that we were dealing with a lower homolog of cholesterol. Naturally occurring $C_{26}$ sterols have recently been encountered[8] among marine sources with the missing carbon atom originating from the side chain. This possibility is clearly excluded because the strong peak at m/e 247 in cholesterol acetate (I) associated (see VI)[6] with the loss of rings A and B is not shifted in the spectrum of the new marine sterol acetate. The one-carbon loss must, therefore, originate from rings A or B, the C-19 angular methyl group being the obvious candidate. The correctness of this assumption was established by comparing the gas chromatographic mobility and mass spectral fragmentation pattern of the P.porosa sterol acetate with those of synthetic 19-norcholesterol acetate (II)[9] which were identical in all respects.

The identification of 19-norcholesterol acetate (II) permits tentative, but reasonable, structure assignments to be made to the other P.porosa sterol acetates, which are less mobile than cholesterol acetate (I). The second acetate (rel. ret. time 1.15) showed M - HOAc (100% rel. int.) and M-[HOAc + $CH_3$] peaks at m/e 368 and 353, identical with cholesterol acetate (I). However, the latter's M - [HOAc + side chain] and M - [HOAc + ring D fission[7]] peaks again were shifted by 14 mass units to m/e 241 and 199 just as had been found with 19-norcholesterol acetate (II). On the other hand, the m/e 247 peak (VI) of cholesterol (I) and 19-norcholesterol (II) acetate had now moved to m/e 261 (VII), thus demonstrating that the new sterol acetate had the ring skeleton of II, but an extra methyl group in the side chain. By analogy to the many naturally occurring

24-methylated marine sterols[10] we assume that the new acetate is 24ξ-methyl-19-norcholesterol acetate (III).

The intense M - HOAc peak of the least mobile sterol acetate occurred at m/e 380, thus requiring two additional carbon atoms and one additional degree of unsaturation as compared to 19-norcholesterol acetate (II). That both compounds possess the identical ring skeleton is shown by the identical m/e 241 (M - HOAc + side chain) and m/e 199 (M - HOAc + ring D fission) peaks now demonstrated to be so typical of 19-nor-$\Delta^5$-3β-acetoxy sterols. The two extra carbons and the double bond of this third sterol must be situated in the side chain because of the prominent m/e 273 peak (VIII), which replaces the m/e 247 (VI) peak of cholesterol (I) and 19-norcholesterol (II) acetate. The double bond is almost certainly located at positions 22 and 23 because of a peak at m/e 337 (M - HOAc + $C_3H_7$) - the $C_3H_7$ grouping originating from carbon atoms 25, 26 and 27. An analogous peak at m/e 351 is present in stigmasterol acetate (IV).[6] Further evidence is provided by the existence of a peak at m/e 239 (M - HOAc + side chain + 2H), a fragmentation typical[11] of unsaturated sterol side chains. On the basis of biogenetic analogy we conclude that the third sterol acetate is 24ξ-ethyl-22-dehydro-19-norcholesterol acetate (V), although a 23,24-dimethyl analog is not excluded by the present evidence.

The three new 19-nor sterol acetates (II, III, V) are easily separated from the more abundant cholesterol acetate (I) present in P.porosa because of their markedly slower mobility during the original separation on $AgNO_3$ impregnated alumina. The absence of the 19-angular methyl group clearly allows for firmer bonding of the $\Delta^5$-double bond to the silver ions.

While synthetic 19-nor steroids are of enormous importance because of their utilization as oral contraceptives, there exists only one published record of naturally occurring 19-nor sterols. Interestingly, this also refers to a marine organism. Minale and Sodano[12] demonstrated the presence in the sponge Axinella polypoides of a series of 19-norstanols, such as the 5,6-dihydro analogs of II, III and V, and especially of 19-nor-5α,10β-ergost-trans-22-en-3β-ol (5,6-dihydro-$\Delta^{22}$ analog of III). Since radioactive acetate was not incorporated by that sponge into sterols, whereas labeled cholesterol was transformed into 19-norstanols, the Italian investigators proposed[13] that these 19-norstanols arose through ring A demethylation by the sponge of dietary cholesterol. While our isolation of 19-nor-$\Delta^5$-stenols from a gorgonian does not shed any light on the question of the exogenous vs. endogenous origin of these sterols among marine animals, it

does demonstrate that whatever the origin, demethylation can precede saturation of the $\Delta^5$-double bond. It is likely that 19-nor sterols will be encountered in many other marine animals; indeed we have recently detected 19-norcholesterol also in Plexaura homomalla. Of particular interest will be the search for possible biogenetic precursors, presumably $\Delta^5$-19-oxygenated marine sterols. If a rich source of 19-nor sterols or appropriate precursors is encountered, then this may also prove to have potential economic significance.

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