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New Evidence for the Structure of Myxinol

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1. Preliminary spectroscopic examination of a second component of hagfish bile salts suggested that it might be 3β,7α,26(27)-trihydroxy-5α-cholestane. 2. Impure reduction products of the 3β,26(27)-dihydroxycholestane-7,16-dione previously made from myxinol disulphate appeared also to have the 5α-configuration. 3. Infrared, nuclear-magnetic-resonance and mass-spectrographic as well as optical-rotatory-dispersion measurements on 3β,26(27)-dihydroxycholestane-7,16-dione showed that it was a 5α-compound. 4. Myxinol is thus 3β,7α,16α,26(27)-tetrahydroxy-5α-cholestane; new nuclear-magnetic-resonance measurements on myxinol tetra-acetate at higher resolution confirm this structure.

The principal bile salt from two species of hagfish, myxinol disulphate, was assigned the structure 3β,7α,16α,26(27)-tetrahydroxy-5β-cholestane 3,26(27)-disulphate (disulphate of I) largely on the basis of physical measurements (Haslewood, 1966; Cross, 1966). Reference was not obtained to any known steroid; nevertheless the structure seemed well supported. In particular, the 5β assignment appeared to agree with the considerable solubility of myxinol digitonide in aqueous ethanol and with interpretation of nuclear-magnetic-resonance (n.m.r.) measurements made on myxinol tetra-acetate.

Two of us (I.G.A. and G.A.D.H.) have isolated a crystalline substance that we believe to be the second, less polar, constituent of hagfish bile salts already mentioned (Haslewood, 1966). We suspected on biogenetic grounds that our new substance might be 16-deoxymyxinol, i.e. on our previously held views of the myxinol formula, 3β,7α,26(27)-trihydroxy-5β-cholestane. However, the infrared (i.r.) spectrum (in potassium bromide) of the new compound showed no resemblance to that of methyl 3β,7α-dihydroxy-5β-cholanoate but resembled so closely that of 3β,7α-dihydroxy-5α-cholestane that we could hardly doubt that we indeed had a substance with a 3β,7α-dihydroxy-5α-cholestane ring nucleus.

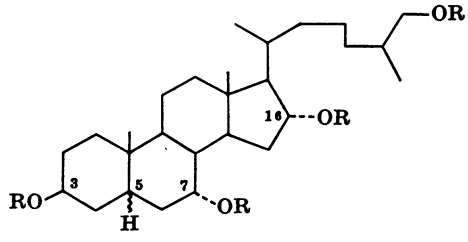
We had also obtained an impure compound by Kishner–Wolff or Raney nickel–ethanedithiol treatment of the dione thought to have structure (IV). Reduction of compound (IV) should give 3β,26(27)-dihydroxy-5β-cholestane and we made this substance by partial synthesis. Although there was a superficial resemblance between our reduction product from the dione and 3β,26(27)-dihydroxy-5β-cholestane, the i.r. spectra were quite different. The i.r. spectrum of our product from myxinol had the principal bands between 6·5 and 11·5μ of 3β-hydroxy-5α-cholestane.

We then suggested to our co-authors (A.D.C. and L.T.) that myxinol might have the 5α-configuration. They had come to the same conclusion by examination of the dione and their report on this and on the n.m.r. spectrum of myxinol tetra-acetate, obtained with an apparatus giving higher resolution than previously possible, follows.

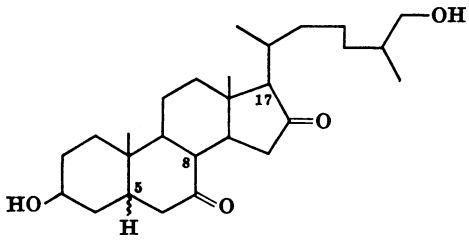
RESULTS AND DISCUSSION

The dioxo oxidation product (V) shows a molecular ion (M^+) at *m/e* 432 in the mass spectrum in agreement with its empirical formula, $C_{27}H_{44}O_4$. The infrared spectrum shows a doublet carbonyl absorption at 1780 and 1710 $cm.^{-1}$, indicating that the carbonyl groups are in a five- and six-membered ring respectively. The location of these carbonyl groups is evident from the mass spectrum, which shows fragmentation products characteristic for both 7-oxocholestane (*m/e* 194 and 207) and 16-oxocholestane (*m/e* 289, 331 and 417). These cleavage patterns are indicated in structure (VI) and their mechanisms have been discussed in detail (Budzikiewicz & Djerassi, 1962; Beugelmans *et al.* 1964; Beard, Wilson, Budzikiewicz & Djerassi, 1964). These fragmentation products are also in agreement with the location of the two hydroxyl groups at C-3 and C-26 or C-27.

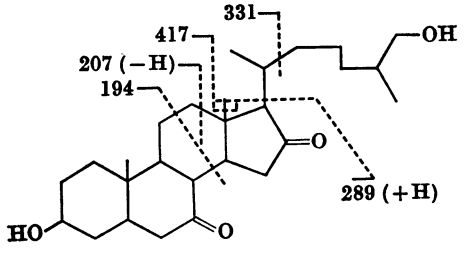
The optical-rotatory-dispersion (o.r.d.) and n.m.r. characteristics of the dione shed further light on the



(I) 5β, R=H
(II) 5α, R=H
(III) 5α, R=CO·CH$_3$



(IV) 5β
(V) 5α



(VI)

stereoconfiguration at C-3, C-5, C-8 and C-17. Both the 7-oxo (Beugelmans *et al.* 1964) and the 16-oxo (Beard *et al.* 1964) steroids are known to retain the 8β- and 17β-configurations respectively under conditions, such as those used in the preparation of the dione, that may cause enolization towards these centres. The 8β and 17β assignments are further substantiated by the o.r.d. and n.m.r. data, but in both cases the data clearly point to a 5α-configuration instead of the previously reported A/B *cis* system.

The very strongly negative Cotton effect in the o.r.d. curve (molecular amplitude −282°) is in agreement with the expected value for a 5α,8β,17β-cholestane-7,16-dione derivative, but its value is too high for a 5β-epimer. The molecular amplitude for 5α-cholestan-16-one is −234° and this value is not influenced significantly by the nature of the side

Table 1. *Calculated (Bhacca & Williams, 1964) and observed C-19 proton resonances for 3,27-dihydroxy-14α,17β-cholestane-7,16-dione*

	Proton resonance (p.p.m.)	
	5α	5β
Androstane	0·79	0·93
17β-Side chain	−0·02	−0·02
16-Oxo*	0·02	0·02
7-Oxo	0·28	0·28
3-equatorial-OH	0·03 (β-OH)	0·01 (α-OH)
Calculated value	1·10	1·22
Observed value	1·12	

*Tori & Aono (1964).

chain (Beard *et al.* 1964). The estimated contribution of the 7-oxo function is positive (+30°) for a 5β-compound (total: −204°) and it is negative (−16°) for the 5α-isomer (total: −250°) (Djerassi & Closson, 1956; Djerassi, Closson & Lippman, 1956). [These reference values were obtained in methanol solution, but there is evidence (Djerassi & Closson, 1956) that the solvent effect is negligible in comparison with the difference between the values for for the 5α- and 5β-isomers.]

The n.m.r. spectrum of compound (V) exhibited two sharp singlet three-proton resonances at 0·80 and 1·12 p.p.m. for the C-18 and C-19 angular methyl groups respectively and two doublets centred at 0·92 and 0·97 (*J* 6·5 and 6·0 cyc./sec. respectively), which, according to the integration of the area, correspond also to three protons each. These resonances can be assigned to the C-21 and C-26 methyl groups coupled with the protons on C-20 and C-25 respectively. The two OH protons appeared at 1·63 p.p.m. as a singlet and the two protons on the hydroxyl-bearing carbon atom, C-26/27, showed a multiplet resonance centred at 3·44 p.p.m. overlapping with the broad resonance between 3·30 and 3·80 p.p.m. (axial hydrogen) due to the proton on C-3. The chemical shift of the C-18 resonance is consistent with the estimated value (0·84 p.p.m.) for a 14α,17β-cholestane-7,16-dione (Bhacca & Williams, 1964; Tori & Aono, 1964). The fact that the position of the C-19 proton resonance is compatible only with the expected value for an A/B *trans* compound (see Table 1) thus provides further evidence for the 5α-configuration.

The axial proton on C-3, with the configuration at C-5 being α, settles then the assignment of the 3-hydroxy group as equatorial, in agreement with the positive digitonin test (Haslewood, 1966).

Reinvestigation of the n.m.r. spectrum of myxinol tetra-acetate (III) on a Varian HA-100 spectrometer showed, in contrast with the earlier spectrum

recorded at 50Mcyc./sec., two sharp three-proton signals at 0·69 (C-18) and 0·83 (C-19) p.p.m. and two three-proton doublets centred at 0·91 and 0·92 p.p.m. (J 7·5 and 5·5cyc./sec. respectively) for the C-21 and C-26 protons. The chemical shift of both the C-18 (Calc. for: 5α, 0·69; 5β, 0·69. Found: 0·69 p.p.m.) and C-19 (Calc. for: 5α, 0·84; 5β, 0·98. Found: 0·83 p.p.m.) signals are now in excellent agreement with the expected values for the 5α-configuration, the C-19 signal being incompatible with the calculated value for the 5β-isomer. The clearly resolved 12-proton signals for the four $-O\cdot CO\cdot CH_3$ methyl groups are at 1·97, 2·01, 2·04 and 2·05 p.p.m.: three protons each. The two protons on C-27 exhibited a multiplet resonance between 3·70 and 4·05 p.p.m., and a broad unresolved signal between 4·50 and 5·05 p.p.m. is due to the protons on C-3, C-7 and C-16.

Thus the two pairs of authors are independently agreed that fresh evidence makes it appear that myxinol is best represented as 3β,7α,16α,26(27)-tetrahydroxy-5α-cholestane (II).

The biological implication is that the 5α (allo) bile salt structure is present in the most primitive extant vertebrate group.

EXPERIMENTAL

The i.r. spectrum was recorded on a Perkin–Elmer 237B spectrometer in KBr. The o.r.d. spectrum was measured in dioxan solution on a JASCO-ORD/UV-5 spectrometer; the n.m.r. spectrum in deuteriochloroform on a Varian HA–100 spectrometer. The chemical shifts (δ) in p.p.m. are measured relative to tetramethylsilane internal standard and are reported to the nearest 0·01 p.p.m. The coupling constants, in cyc./sec., are reported to the nearest 0·5cyc./sec. The mass spectrum was recorded with an Atlas CH–4 mass spectrometer, equipped with a TO–4 ion source. The ionizing energy was maintained at 70ev and the ionizing current at 10μA.

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