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# MINOR AND TRACE STEROLS IN MARINE INVERTEBRATES 40.[1] STRUCTURE AND SYNTHESIS OF AXINYSSASTEROL, 25-METHYLFUCOSTEROL AND 24-ETHYL-24-METHYLCHOLESTEROL -- NOVEL SPONGE STEROLS WITH HIGHLY BRANCHED SIDE CHAINS

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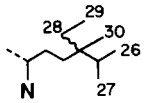
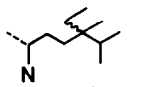
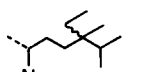
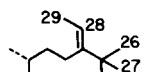
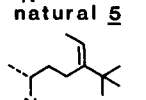
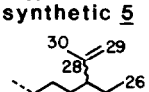
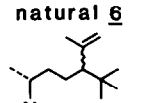
Abstract: The isolation, structure determination and synthesis of three novel sterols with unusual side chains is reported. A consistent scheme is proposed which accounts for the biosynthesis of all major and minor sterols in this sponge.

Recently,[3-5] we recorded the structure elucidation of several marine sterols with highly branched side chains, which represent the most exotic array of biomethylation sequences hitherto encountered in the steroid field. These structures are all consistent with a plausible biosynthetic scheme,[6] starting with epicodisterol, which, however, has not yet been confirmed experimentally by tracer incorporation studies. We have now solved[7] the technical problem of incorporating a sterol precursor (such as epicodisterol) into a sponge and, therefore, are searching for the best substrates in which the largest number of unusual biomethylations can be demonstrated experimentally in one animal. In this paper we report our isolation and structure eludication studies with a Pseudoaxinyssa sp. (Australian Great Barrier Reef) which appears to be an extraordinarily interesting candidate, since it is virtually devoid of conventional sterols.

Hofheinz and Oesterhelt[8] reported that this sponge[9] contained only 24-isopropylcholesterol (1) and its 22-dehydro analog 2. Together with 24-isopropylidenecholesterol (3),[10] these are the only natural marine sterols with a 24-isopropyl substituent. This raises interesting questions concerning their biosynthesis and biological function and we considered it worth while to look for trace sterols, which might represent biosynthetic markers. Separation of the sterol mixture by reverse phase HPLC (Altex Ultrasphere ODS-5 with 98:2 MeOH-$H_2O$) provided 47% of 1,[8] 51% of 2[8] and 1.5% of 3[9] as well as three new trace sterols,[11] whose properties and synthesis are herewith reported.

24-Ethyl-24-methylcholesterol (4) [rel. ret. time: GC, 2.23 (3% OV-17); HPLC, 1.25 (ODS-2)] is one of the few known sterols[3-5] with a quaternary center at C-24. The $^1H$ NMR data (Table 1) are consistent with the assigned side chain substitution pattern. The characteristic mass spectral peaks at m/z 428.403 ($M^+$, $C_{30}H_{52}O$), 273, 255, 231 and 213 demonstrate the presence[12] of the conventional cholesterol nucleus and the absence[13] of unsaturation in the side chain. The complete structure (except for C-24 stereochemistry) was proved by synthesis involving LDA-promoted

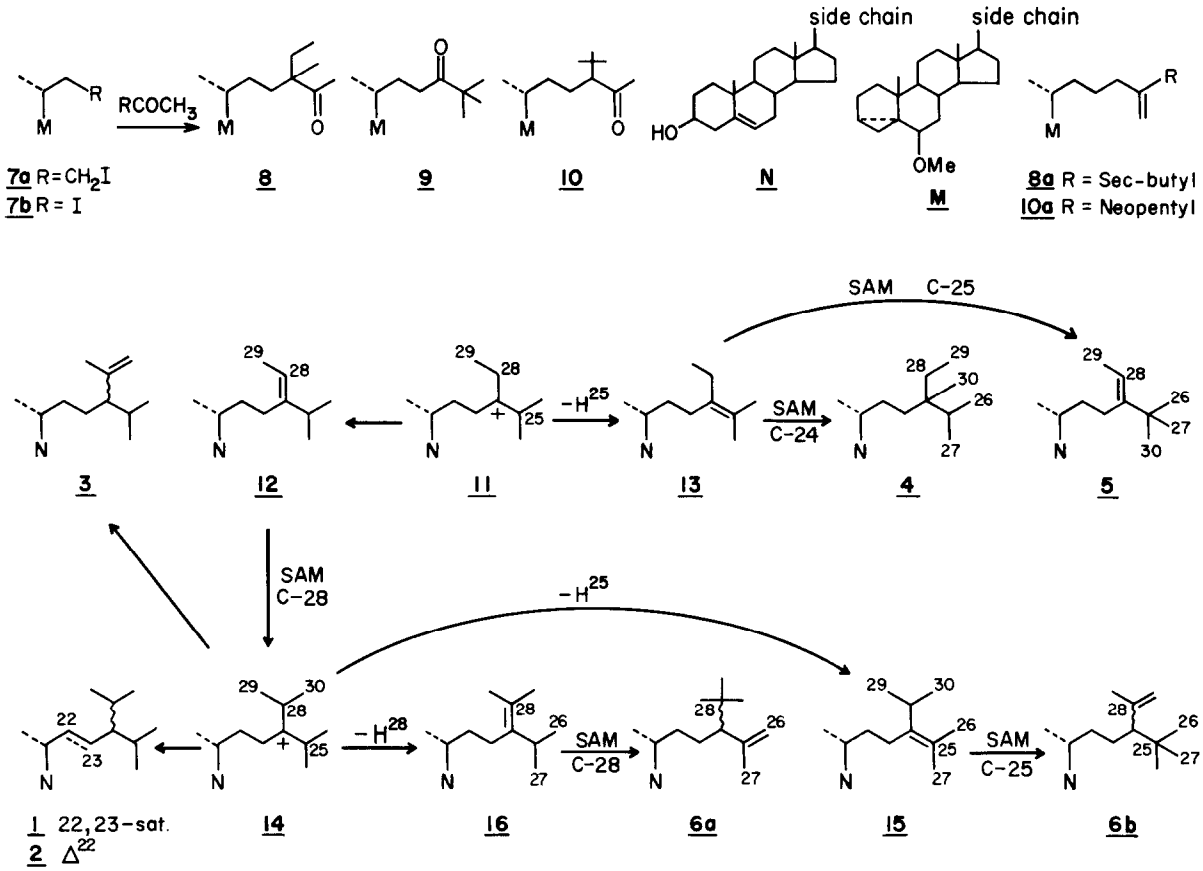
Table 1. Selected $^1$H NMR Chemical Shifts ($C_6D_6$, 360 MHz) of 24-methyl-24-ethylcholesterol (4), 25-methylfucosterol (5) and axinyssasterol (6)

| | Me | | | | | | | | H | |
|---|---|---|---|---|---|---|---|---|---|---|
| | C-18 | C-19 | C-21 | C-26 | C-27 | C-29 | C-30 | C-31 | C-28 | C-29 |
|  natural 4 | 0.671 | 0.950 | 1.026 (d) J=6.54 | 0.858 (d) J=6.82 | 0.869 (d) J=6.90 | 0.835 (t) J=7.47 | 0.767 | | | |
|  synthetic 4 | 0.672 | 0.951 | 1.024 (d) J=6.42 | 0.852 (d) J=6.95 | 0.875 (d) J=6.62 | 0.828 (t) J=7.26 | 0.766 | | | |
|  synthetic 4 | 0.672 | 0.951 | 1.025 (d) J=6.61 | 0.857 (d) J=6.87 | 0.869 (d) J-6.85 | 0.834 (t) J=7.40 | 0.766 | | | |
|  natural 5 | 0.671 | 0.950 | 1.079 (d) J=6.44 | 1.124 | 1.124 | 1.675 (d) J=6.60 | 1.124 | | 5.412 (q) J=6.75 | |
|  synthetic 5 | 0.671 | 0.950 | 1.079 (d) J=6.44 | 1.124 | 1.124 | 1.675 (d) J=6.60 | 1.124 | | 5.412 (q) J=6.72 | |
|  natural 6 | 0.669 | 0.946 | 1.053 (d) J=6.60 | 0.961 | 0.961 | | 1.703 | 0.961 | | 4.798 (1H) 4.962 (1H) |
|  synthetic 6 | 0.671 | 0.948 | 1.050 (d) J=6.63 | 0.959 | 0.959 | | 1.704 | 0.959 | | 4.795 (1H) 4.960 (1H) |

(THF, 0°, 2 hr., $N_2$) condensation of the iodide 7a[14a] with methyl sec.-butyl ketone, separation (reverse phase HPLC, Whatman Partisil M9 10/50 ODS-2, MeOH) of the desired ketone 8 from the isomer 8a, Wittig condensation (butyl lithium, THF, 20 hr., reflux), hydrogenation ($PtO_2$, 18 hr., EtOAc) of the resulting methylene double bond and removal of the i-methyl ether protecting group. The two C-24 isomers were separated chromatographically at the ketone 8 stage (Altex Ultrasphere ODS-5, 95:5 MeOH-$H_2O$) and then carried separately through the last three steps. As shown in Table 1, the NMR properties of one of the two C-24 stereoisomers (identical GC and HPLC r.r.t.) were completely identical with those of the natural sterol 4.

The structure elucidation of 25-methylfucosterol (5) ($M^+$ 426; r.r.t.: GC, 2.10; HPLC, 1.10) was straightforward. In addition to the conventional mass spectral peaks (m/z 271, 253, 229, 213) associated with the cholesterol nucleus,[12] an intense peak at m/z 314 due to a McLafferty rearrangement[15] of the $\Delta^{24(28)}$-double bond was observed. The NMR spectral data (Table 1) immediately pointed to structure 5, which was verified by synthesis through condensation (LDA, 0°, 2 hr., $N_2$) of the iodide 7b[14b] with methyl tert.-butyl ketone to the steroidal ketone 9, Wittig

condensation (butyl lithium, benzene, 24 hr., reflux) with triphenylethylphosphonium bromide and removal of the ring-A protecting group. While the yield of the desired Wittig condensation product was poor (15%), only one stereoisomer was obtained, which points to the E stereochemistry of the double bond.




The molecular ion ($M^+$ 440, $C_{31}H_{52}O$) of the third trace sterol together with the mass spectral peaks[12] of the conventional cholesterol nucleus demonstrated that the sterol had to be the product of a rare[3-5] quadruple biomethylation sequence in the side chain. The presence of a $\Delta^{25}$-double bond followed from the diagnostic[16] peaks at m/z 328, 314 and 299, while the existence of a tert.-butyl substituent was based on the nine-proton NMR singlet at $\delta$ 0.959 (Table 1). Its attachment at C-24 was suggested by a significant mass spectral peak at m/z 384 ($M^+ - C_4H_8$) arising from an alternative McLafferty rearrangement. The resulting structure 6 was confirmed by condensation of the iodide 7a[14a] with methyl neopentyl ketone to the ketone 10 (cleanly separated by HPLC from the major product 10a) followed by Wittig condensation with triphenylmethylphosphonium bromide and deprotection. We assign the trivial name axinyssasterol rather than a

systematic name to 6, because the sterol can be depicted as either 6a or 6b with differing biosynthetic implications.

The virtually exclusive existence[11] of the uniquely branched sterols 1-6 can be accommodated in a single biosynthetic scheme from the expected carbocation 11 due to conventional SAM (S-adenosylmethionine) attack on 24-methylenecholesterol.[17] Our isolation of 4 and 5 strongly points to the intermediacy of 24-ethyldesmosterol (13). The other four sterols (1,2,3,6) can all be derived from the cation 14 resulting from the hitherto unknown SAM attack at C-28 of (iso)-fucosterol (12). All of these predictions can ultimately be tested by administering labelled 12 and 13 to the sponge. By suitable placement of the label, it should be possible to differentiate between the structurally identical but biosynthetically different representations 6a and 6b for axinyssasterol.[18]

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REFERENCES

1. For preceding paper, see C. A. N. Catalan, V. Lakshimi, F. J. Schmitz and C. Djerassi, Steroids, in press.
2. Visiting investigator from Shenyang College of Pharmacy and Liaoning Institute of Materia Medica, People's Republic of China.
3. L. N. Li and C. Djerassi, J. Am. Chem. Soc., 103, 3606 (1981).
4. L. N. Li, U. Sjöstrand and C. Djerassi, J. Org. Chem., 46, 3867 (1981).
5. L. N. Li and C. Djerassi, Tetrahedron Lett., 22, 4639 (1981).
6. C. Djerassi, Pure and Appl. Chem., 53, 873 (1981).
7. C. A. N. Catalan, J. Thompson, W. C. M. C. Kokke and C. Djerassi, in preparation.
8. W. Hofheinz and G. Oesterhelt, Helv. Chim. Acta, 62, 1307 (1979).
9. P. R. Bergquist, W. Hofheinz and G. Oesterhelt, Biochem. Syst. Ecol., 8, 423 (1980).
10. W. C. M. C. Kokke, C. S. Pak, W. Fenical and C. Djerassi, Helv. Chim. Acta, 62, 1310 (1979).
11. Traces (<0.1%) of stigmasterol, stigmastanol and 24-isopropylcholestanol-3β-ol -- all of them with undetermined configuration at C-24 -- were also encountered.
12. S. G. Wyllie, B. A. Amos and L. Tökes, J. Org. Chem., 42, 725 (1977).
13. S. G. Wyllie and C. Djerassi, J. Org. Chem., 33, 305 (1968).
14. (a) N. Theobald, R. J. Wells and C. Djerassi, J. Am. Chem. Soc., 100, 8010 (1978). (b) J. J. Partridge, S. Faber and M. R. Uskokovic, Helv. Chim. Acta, 57, 764 (1974).
15. I. J. Massey and C. Djerassi, J. Org. Chem., 44, 2448 (1979).
16. C. Djerassi, Pure and Appl. Chem., 50, 171 (1978).
17. E. Lederer, Quart. Rev. Chem. Soc., 23, 453 (1969); W. R. Nes and M. L. McKean, "Biochemistry of Steroids and Other Isopentenoids", University Park Press, Baltimore, 1977, ch. 9.
18. Bergquist et al. (ref. 9) suggested on the basis of the mass spectrum of the TMS derivative that an unknown sterol in the sponge Hymeniacidon perleve may be 24-tert.-butyl-22-dehydrocholesterol. Unfortunately, insufficient material was available for NMR measurements.
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