Tetrahedron Letters No. 45, pp 4369 - 4372. 0040-4039/78/1101-4369$02.00/0

DETERMINATION OF THE ABSOLUTE CONFIGURATION OF STELLIFERASTEROL AND STRONGYLOSTEROL - TWO MARINE STEROLS WITH "EXTENDED" SIDE CHAINS

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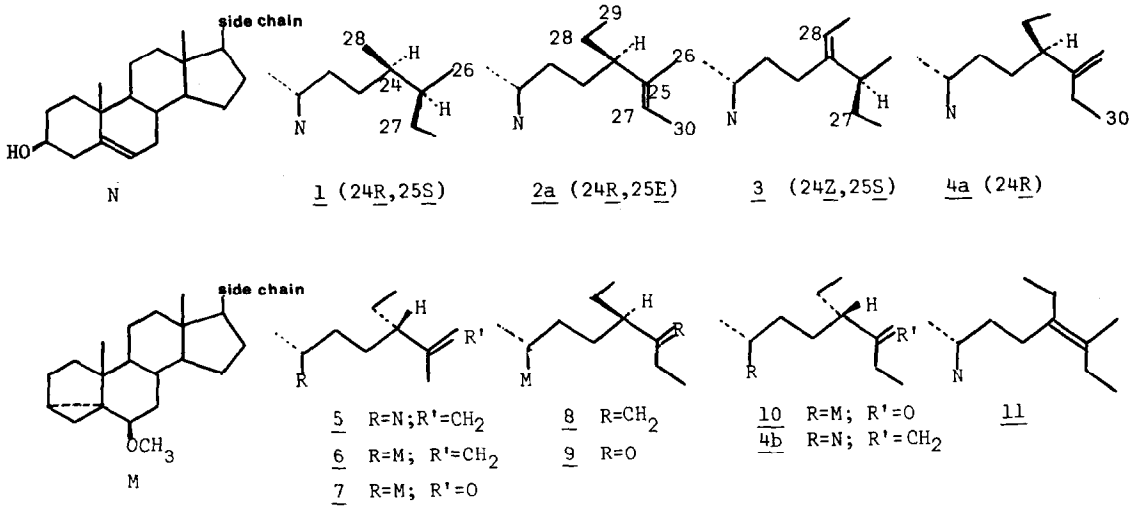
The unusual side chains encountered in recent years[1] among marine sterols may ultimately prove to be of considerable significance in determining the role of sterols, other than cholesterol, in membrane function.[2] Of particular interest in that regard are the uniquely marine sterols with "extended" side chains and their possible biosynthetic origin. Aplysterol (1) and its 24-28 dehydro analog have until recently been the only sterols[3] with "extended" side chains, i.e. with an extra carbon atom attached to C-27. Since then, we reported[4] the isolation and structure elucidation of stelliferasterol (2a) and isostelliferasterol (3) - two marine sterols in which for the first time bioalkylation at C-27 and C-28 has been demonstrated - and shortly thereafter, Tursch, et al.[5] encountered a third member of this class, strongylosterol (4a). The latter is the sole sterol of the sponge Strongylophora purissima and in the other examples cited,[3,4] various sterols with "extended" side chains were the principal sterol constituents. It is likely, therefore, that they play a biological (membrane function?) role rather than being metabolic "dead ends". We have already pointed out[4,6] that several biosynthetic routes can be envisaged[7] that would lead to alkylation at the terminus of the cholesterol side chain, but for the design of the proper isotopically labelled precursors, one must know the absolute configuration at C-24. This had been established as 24R in aplysterol (1) by X-ray analysis,[8] but was unknown in stelliferasterol (2a) and strongylosterol (4a). We now report the successful resolution of this problem by a combined chemical-spectral method which is of general applicability.[9]

Goad and collaborators[10] have distinguished between epimeric C-24 alkylated cholesterols by NMR spectroscopy. However, as the effect of a Δ^{25} double bond (cf. 2 and 4) on these very small ($\Delta\delta$=0.006-0.01 ppm) chemical shift differences of such epimers was unknown, it was first necessary to have available a reference standard. Therefore, natural[11] clerosterol (5), of known[12] (24S) absolute configuration, was subjected successively to i-methyl ether formation (6), ozonolysis (7), Wittig condensation (n-butyl lithium, THF, 48 hr, reflux) and regeneration of the Δ^5-3β-hydroxy moiety to afford a readily separable (reverse phase HPLC) mixture of the 25E (2b) and 25Z (2d) isomers with the 24S configuration. The 360 MHz NMR spectra (Table 1) confirm the absence of epimerization at C-24 during the ozonolysis and Wittig condensation steps. Since earlier NMR measurements[4] had established the 25E stereochemistry for the side chain double bond of stelliferasterol, the NMR data (Table 1) demonstrate that the naturally occurring sterol (2a) is epimeric at C-24 with the synthetic 24S,25E isomer 2b and hence has the 24R,25E absolute configuration.

The key NMR conclusion (Table 1) can be summarized by stating that in the 24S series, the C-29 methyl signal is found downfield and the C-21 methyl resonance upfield compared to the 24R epimer. However, since the chemical shift differences between the epimers are quite small ($\Delta\delta$=0.007 - 0.010 ppm), it is necessary to know the shifts of both isomers to be able to determine the configuration of a sterol with unknown C-24 stereochemistry. In order to apply this finding to the establishment of the C-24 configuration of strongylosterol (4a), we synthesized both C-24 epimers (4a and 4b) of strongylosterol by applying the above described reaction sequence (i-ether formation, ozonolysis to nor ketone and reconstitution of side chain by Wittig condensation) to strongylosterol (4a) itself. The only difference was the insertion, after ozonolysis of strongylosterol i-methyl ether (8) to the 26-nor ketone 9, of a base equilibration step (methanolic sodium methoxide, 1.5 hr, 70°C) so as to yield a mixture of C-24 epimeric ketones (9 and 10) and thence of C-24 epimers of strongylosterol (4a and 4b). The data in Table 2 demonstrate that while the high resolution NMR spectrum of strongylosterol (4a) alone provides no information concerning its C-24 stereochemistry, comparison with the relevant NMR chemical shifts of the mixture of synthetic isomers (4a,4b) clearly shows that the natural sterol has the 24R stereochemistry because of an upfield C-29 and a downfield C-21 methyl signal. This assignment was confirmed by NMR measurements (Table 2) in the 26-nor ketone series; the 29-methyl signal of the 24S-ketone 7, derived from clerosterol (5), displayed a downfield shift compared to its epimeric mixture (obtained by base equilibration of 7), whereas the ketone 9 derived from strongylosterol (4a) showed a high-field C-29 methyl signal and hence belonged to the 24R series.



Our results show that NMR examination of the 26-nor ketones has advantages when only the natural sterol is available, since 3-5 mg is sufficient for the i-methyl ether protection, ozonolysis and base equilibration steps. However, NMR measurements on the parent sterol[10] are preferable when both C-24 isomers are available as, for instance, by synthesis.

Our demonstration of the 24R configuration in stelliferasterol (2a) and strongylosterol (4a) makes it unlikely[13] that clerosterol (5) with its 24S configuration is their biogenetic precursor

by the usual bioalkylation[14] via S-adenosylmethionine and proton loss. The hitherto unknown 24R epimer of clerosterol (5) would be the most attractive candidate and the fact that it has not been described so far does not mean that it does not exist. Epimers at C-24 cannot be distinguished by GC in the usual GC-MS analysis of marine sterol extracts and it is clear that in the future the absolute configuration of asymmetric centers in the sterol side chain will have to be examined more carefully by isolation of sufficient material to subject it to the type of NMR analysis[10] described herewith. A pertinent example is recorded in the accompanying paper.[9]

Table 1. NMR Chemical Shifts of Stelliferasterol (2a) and Isomers ($CDCl_3$, 360 MHz)

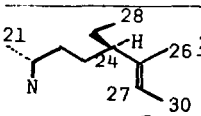
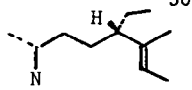
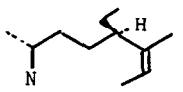
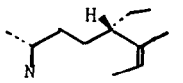
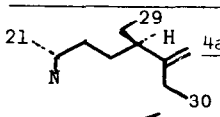
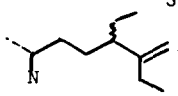
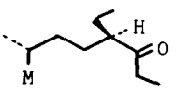
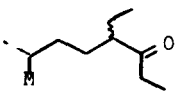
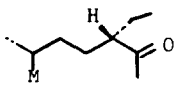
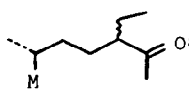
	C-18-Me	C-29-Me	C-21-Me	C-19-Me	C-26-Me	C-30-Me	C-27-H
 2a(24R,25E)	0.659	0.748	0.899	1.003	1.422	1.569	5.153
 2b(24S,25E)	0.667	0.756	0.892	1.005	1.418	1.568	5.158
 2c(24R,25Z)	0.666	0.780	0.912	1.005	1.495	1.544	5.303
 2d(24S,25Z)	0.667	0.785	0.903	1.004	1.499	1.555	5.303

Table 2. NMR Chemical Shifts of Strongylosterol (4a) and Related Compounds ($CDCl_3$, 360 MHz)

	C-29-Me	C-21-Me	C-30-Me
 4a(24R)	0.795	0.907	1.028
 4a,b(synth.mixt.)	0.794 0.799	0.906 0.899	1.029
 9(24R)(ex 4a)	0.835	0.902	1.041
 9,10(synth.mixt. ex 9)	0.835 0.846	0.903	1.041
 7(24S)(ex 5)	0.865	0.911	-
 osynth.mixt.(ex 7)	0.855 0.866	0.912	-

Acknowledgment: We are indebted to our colleague Dr. W. Kokke and to Prof. B. Tursch and collaborators (University of Brussels) for samples of clerosterol and strongylosterol respectively. Financial support from the National Institutes of Health (grants No. GM-06840 and AM-04257) is gratefully acknowledged as is access to the 360 MHz NMR spectrometer facility funded by the National Science Foundation (GP-23633) and the National Institutes of Health (grant No. RR-0711).

References and Notes

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(Received in USA 15 August 1978)