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IDENTIFICATION OF C-24 ALKYLATED STERANES BY P.M.R. SPECTROSCOPY

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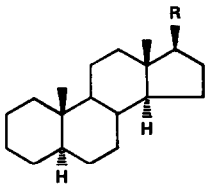
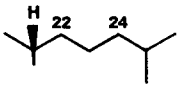
A question of considerable biosynthetic and taxonomic importance is the stereochemistry of the C-24 alkylated sterols found in many species[1]. No method of chromatographic separation of C-24 epimers has yet been found[2]. The stereochemistry of some Δ^{22} unsaturated sterols has been determined by ozonolysis and optical measurements on side-chain fragments[3], though this method may indicate only the predominant epimer because of possible partial racemisation by enolisation of the intermediate aldehyde. The configuration of sidechain-saturated sterols has been assigned by interconversion and α_D determination[4]. However, such optical measurements require high purity and accuracy, while the quantity of material available is often small. High resolution proton magnetic resonance (PMR) spectroscopy provides a more reliable analysis of side chain stereochemistry.

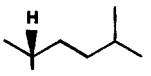
Early investigations of the PMR spectra of sterols demonstrated that useful structural information can be gained from methyl group chemical shifts, particularly C-18 and C-19[5]. However, detailed investigation of the sidechain methyl signals of alkylated sterols has been reported only recently[6]. Significant differences between the spectra of C-24 epimeric pairs at both 60 and 100 MHz were demonstrated. Our studies on the parent hydrocarbons at 100 and 220 MHz reveal that in many steranes the terminal methyl groups exhibit magnetic non-equivalence which is sensitive to configuration. Also the reported peak assignments for the alkylated steroids are revised. The potential of this method of analysis is demonstrated by investigation of the sterols of soya bean and Zea mays.

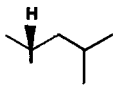
The PMR spectra of 5α-cholestane (1) show equivalent 26 and 27-methyl groups in several solvents. However, in 24-dimethyl-5α-cholane (2) two doublets are observed for the terminal methyls in both $CDCl_3$ and C_6D_6 [Table 1]. A larger chemical shift difference ($\Delta\delta$) is observed in the homologue 23-methyl-5α-cholane (3) and in 20-methyl-5α-pregnane (4). The long-range induction of non-equivalence in prochiral side-chain groups is further demonstrated by 24-diethyl-5α-cholane (5), where two triplet methyls are observed though the protons are separated by six σ bonds from the nearest chiral centre. The contributions of intrinsic non-equivalence[7] and conformational effects to $\Delta\delta$ are uncertain.

The additional chiral centre in C-24 alkylated steranes might be expected to enhance chemical shift differences in the terminal methyls and provide a sensitive method of distinguishing between isomers. The spectra of 5α-stigmastane (6) and 5α-poriferastane (7) are significantly different in both $CDCl_3$ and C_6D_6 at 100 MHz. Analysis of line intensities suggested non-equivalence of the 26 and 27 methyls. This was confirmed by the spectra



(1) R = 

(2) R = 

(3) R = 

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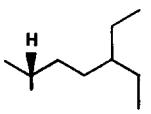
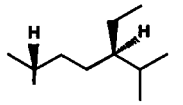
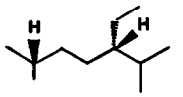
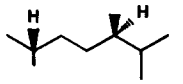
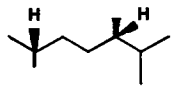
(5) R = 

(6) R = 

(7) R = 

(8) R = 

(9) R = 

of their 5β isomers and by the 220 MHz spectra of (6) and (7), [Table 1]. The diagnostic value of the slight chemical shift differences is illustrated in the Figure. As an adjunct to the PMR study the ORD spectra of the compounds were measured. All gave plain positive curves. The additive effect of the C-24 chiral centre is demonstrated by calculating the $\Delta[\Phi]$ values relative to cholestane at various wavelengths. Values for (6) and (7) are symmetrically distributed about that of cholestane [Table II].

The 100 MHz spectra of 5α-ergostane (9) showed unresolved methyl signals, the previous assignments of which[6] seemed incompatible with the observed line intensities. An interpretation involving non-equivalence of the terminal methyls was confirmed at 220 MHz [Figure]*. 5α-Campestane (8), derived from commercial soya-bean "sitosterol", showed unexpectedly complex 100 MHz spectra. Also the ORD data gave less positive $\Delta[\Phi]$ values than predicted from 5α-ergostane [Table II]. The 220 MHz spectra revealed the samples to be mixtures (~70:30) of campestane:ergostane [Figure]*. This ratio is confirmed by the ORD data and by the spectra of 5β-"campestane" and a 1:1 mixture of "campestane" and ergostane. The presence of a mixture of isomeric C-28 sterols has also been demonstrated for Zea mays, inferring a hitherto unsuspected complexity in plant sterol biosynthesis, which is being further investigated.

Thus, the stereochemistry of C-24 alkylated steroids can be deduced unambiguously from the 220 MHz PMR spectra of the derived steranes. The composition of epimeric mixtures can also be estimated semi-quantitatively.

We thank Dr. G. Ryback for ORD spectra, Mr. R. Carrington and Dr. D. Clague for PMR spectra and Dr. L. J. Goad for a gift of poriferasterol.

* Exact assignments were made from the spectra of 24,28-$^{2}H_{2}$-24 methyl-5β-cholestane.

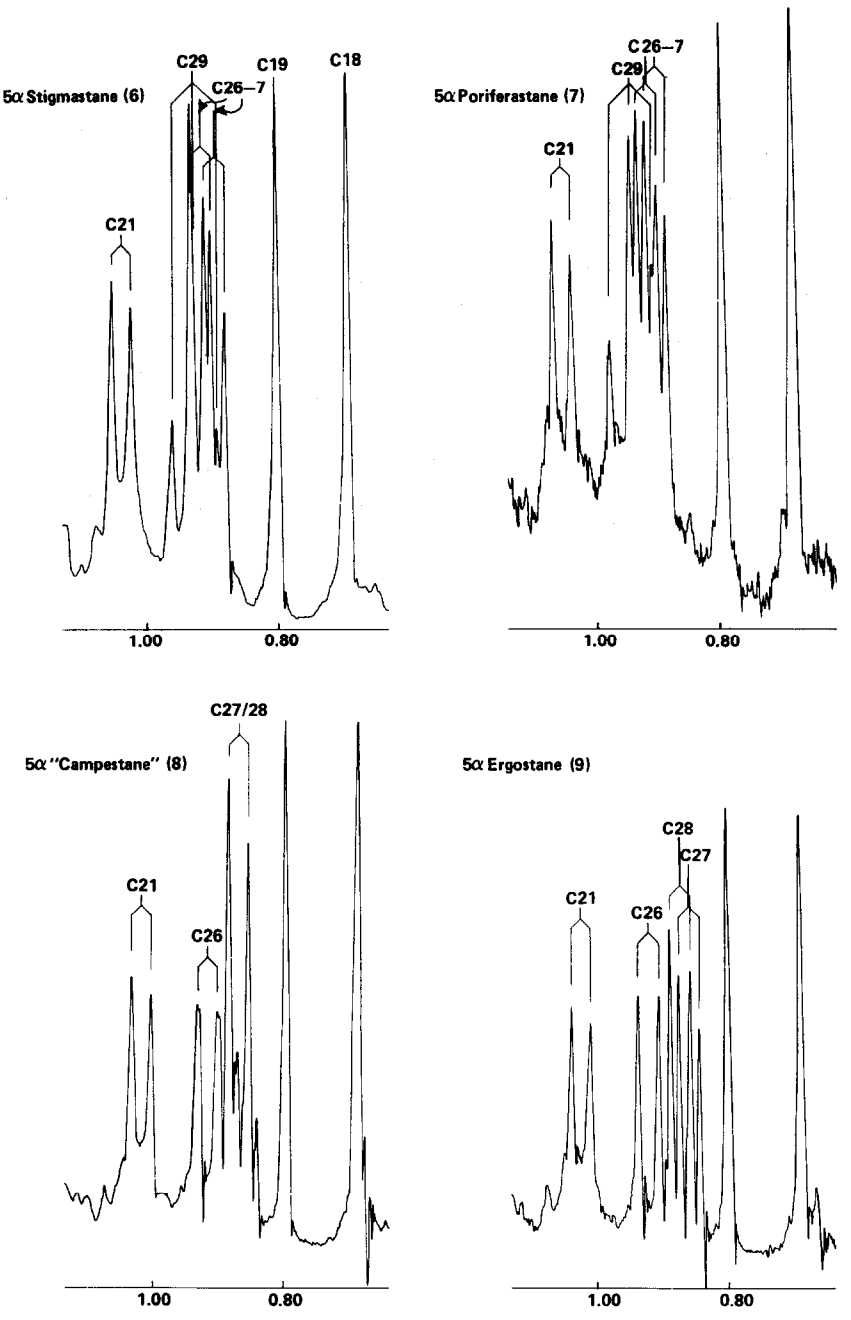


Figure. High-field region of 220MHz PMR spectra of steranes in C_6D_6 (ca. 0.8%) at ambient temperature

TABLE I. Chemical Shifts of Sterane Methyl Groups[a]

Compound	$CDCl_3$					C_6D_6				
	C-21	C-28(9)	Terminal methyls		Δδ	C-21	C-28(9)	Terminal methyls		Δδ
(1)[b]	0.897	-	0.861		0.0	1.015	-	0.928		0.0
(2)[b]	0.890	-	0.862	0.849	0.013	0.994	-	0.940	0.935	0.005
(3)[b]	0.867	-	0.867	0.805	0.062	0.972	-	0.944	0.885	0.059
(4)[b]	-	-	0.919	0.830	0.089	-	-	0.983	0.894	0.089
(5)[c]	0.898	-	0.828	0.818	0.010	1.033	-	0.914	0.905	0.009
(6)[c]	0.904	0.841	0.832	0.808	0.024	1.038	0.927	0.917	0.896	0.021
(7)[c]	0.905	0.848	0.826	0.804	0.022	1.047	0.940	0.915	0.901	0.014
(8)[c,d]	0.891[e]	0.768	0.848	0.796[f]	0.052	1.019	0.870	0.915[g]	0.870[h]	0.045
(9)[c]	0.900	0.770	0.850	0.777	0.073	1.024	0.871	0.920	0.858	0.062

a. Values in p.p.m from TMS at concentrations of c.a. 0.8%; b. 100 MHz spectra; c. 220 MHz spectra; d. Data for major component, campestane; minor component peaks at:- e. 0.898δ, f. 0.776δ, g. 0.918δ, h. 0.857δ.

TABLE II. Values of $\Delta[\phi]$ at various wavelengths[a.b.]

Compound	Wavelength (nanometers)			
	233	286	400	588
(6)	+179	+ 82	+ 30	+ 11
(7)	-172	- 73	- 28	- 10
(8)	+119	+ 63	+ 29	+ 12
(9)	-452	-212	- 82	- 32

a. $\Delta[\phi] = [\phi]$ compound - $[\phi]$ cholestane,
b. concentration 0.16 - 0.56% in hexane.

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