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THE 220 MHz NMR SPECTRA OF PHYTOSTEROLS

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Abstract—The 220MHz NMR spectra of forty two steroids are reported. Eight pairs of C-24 epimers (24α- and 24β) and two pairs of double bond isomers (*cis* and *trans*) can be distinguished by this technique. The influence of substituents, solvents and stereochemistry on methyl group chemical shifts is discussed.

INTRODUCTION

Many phytosterols contain an alkyl group at C-24 and several epimeric pairs of sterols are known [1]. In general, algae [2] and fungi produce sterols with the 24β-configuration§ while in most higher plants the sterols have the 24α-configuration [1]. The C-24 alkyl group is derived biosynthetically by a transmethylation reaction from S-adenosylmethionine and several mechanisms have been elucidated using different classes of plants [6,7]. Since the C-24 configuration and alkylation mechanism may have some phylogenetic significance [7] it is important to be able to establish the C-24 stereochemistry of phytosterols reliably. Also many marine invertebrates contain complex sterol mixtures which probably include phytosterols of dietary origin [8–10]. Precise identification of these compounds may give an indication of the primary plant source [11].

Chromatographic separation of pairs of C-24 epimeric sterols has not been reported, nor can they be differentiated by IR or MS. In the case of Δ^{22} unsaturated sterols determination of stereochemistry has involved sidechain cleavage by ozonolysis and measurement of optical rotation on derivatives of the resulting aldehyde or acid [12,13] though this method only determines the predominant stereochemistry, since considerable racemisation of the ozonolysis product is often encountered. For sterols containing saturated sidechains, assignments have been based on specific rotation difference at fixed wavelengths and on melting points [14,15]. However, in some epimer pairs these differences are small. In addition, such measurements can give only the predominant stereochemistry if both epimers are present, or alternatively, the purification procedures, such as recrystallisation, which are necessary before optical or melting-point measurement can be made, could result in removal of one epimer from a mixture prior to analysis.

Recently ^{1}H-NMR spectroscopy has been used to assign C-24 stereochemistry. The 100 MHz spectra of some epimeric pairs of sterols show distinct differences in the methyl group patterns [5]. The 220 MHz spectra of C-24 alkylated steranes give unique methyl group patterns for each epimer [16], facilitating unambiguous assignment of chemical shifts and coupling constants. Increased resolution of the signals also permits semiquantitative analysis of mixtures of epimers. We have used 220 MHz spectroscopy to characterise sterols and now report the NMR spectra of a number of compounds including C-24 isomeric pairs [17], to illustrate the scope of this method of stereochemical assignment.

The pattern of signals observed in the high-field region of many phytosterol spectra reveals non-equivalence of the two methyl groups of the *iso*-propyl moiety [18]. The bulk of the sidechain substituents is insufficient to cause hindered rotation about the C-24/25 bond at normal temperatures. Therefore, the observed difference in

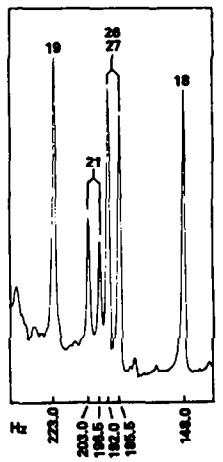

Fig. 1. Methyl group region of 220 MHz NMR spectrum of cholesteryl acetate ($CDCl_3$).

§ The configuration at C-24 can be specified by either the 24α- and 24β- or by the (24*R*)- and (24*S*)- nomenclature. The latter is recommended by the IUPAC/IUB Rules [3]. However, the introduction of a Δ^{22} bond changes the priorities of the groups attached to C-24 and so a (24*R*)-saturated sidechain becomes a (24*S*)-Δ^{22} sidechain [1]. For clarity the 24α- and 24β-system has been adopted in the text as recommended by other authors [4,5]. The trivial names and systematic names (with *R* and *S* nomenclature) of the sterols are listed in the Experimental. Full structures are given in ref. [1].

chemical shift between the 26/27-methyl group doublets results mainly from *intrinsic magnetic non-equivalence* generated by the C-24 chiral centre [18]. Also, the spectra of steranes bearing modified sidechains [16] suggest that this non-equivalence is further modulated by the influence of the chiral steroid ring system. Such an effect could result in slight differences in chemical shift of 26- and 27-methyls between epimers. In addition, the normal diastereomeric relationship between the chiral centres at C-20 and 24 might be expected to produce chemical shift differences in both the C-21 and C-28(9) methyl signals of epimeric pairs of sterols.

In view of the involvement of the nucleus:sidechain interaction in creation of these diagnostic patterns of methyl group signals, we have examined the effects of ring and sidechain substituents on the analysis. The utility of an alternative aromatic solvent is also illustrated and limitations of this method of stereochemical assignment are discussed.

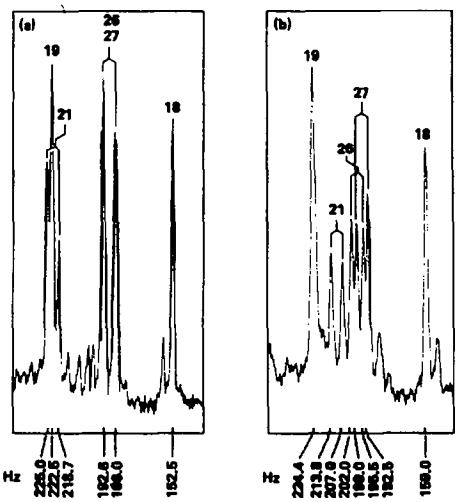

Fig. 2. 220 MHz NMR spectra of (a) cholesta-5,*E*-22-dien-3β-ol and (b) cholesta-5,*Z*-22-dien-3β-ol ($CDCl_3$).

RESULTS AND DISCUSSION

The NMR spectrum of cholesteryl acetate, presented for comparison purposes (Fig. 1), shows the expected chemical shifts for the singlets due to the protons of the C-18 and C-19 methyl groups, a doublet for the C-21 methyl protons and a doublet for the equivalent C-26 and C-27 methyl protons. In the spectrum of 5α-cholest-7-en-3β-yl acetate the C-18 and C-19 methyl proton resonances are displaced upfield as expected [19] for a Δ^7 sterol (Table 1). Cholesta-5,*E*-22-dien-3β-ol and cholesta-5,*Z*-22-dien-3β-ol have strikingly different spectra (Fig. 2a and 2b). With the Δ^{22}-trans compound (Fig. 2a) the C-18 methyl singlet and the C-21 methyl doublet are downfield compared to the corresponding resonances of cholesterol, due to the influence of the Δ^{22}-bond. There is also one doublet for the equivalent C-26 and C-27 methyl groups. The spectrum of the Δ^{22}-*cis* compound (Fig. 2b) reveals a further significant downfield shift for the C-18 methyl resonance, as reported previously in the 100MHz NMR spectrum [20]. However, more notable is the upfield shift of the doublet for the C-21 methyl protons and the appearance of a pair of doublets for the protons of the C-26 and C-27 methyls which are now nonequivalent. The IR spectra of Δ^{22}-sterols have been used to differentiate Δ^{22}-*cis* and *trans* isomers, but, as this example shows, the NMR spectrum also provides a powerful method for assignment of Δ^{22}-configuration.

The 24-ethylidene sterols, fucosterol and 28-isofucosterol, can be readily differentiated by their 100MHz spectra, where the shift of the C-25 proton is further downfield in the latter compound [21,22]. This is also seen [23] in the 220MHz spectra of these compounds but other differences are also apparent in the chemical shifts of the methyl group protons (Table 1). In the *E*-isomer the C-18 singlet and the doublet for the equivalent C-26 and C-27 protons are downfield (deshielded 0·02 ppm) and the C-21 doublet upfield (0·09ppm) compared to the corresponding signals in the spectrum of the *Z*-isomer.

24-methylsteroids

The methyl group chemical shifts of a series of 24-methyl steroids are listed in Table 2 and the spectra of some epimeric pairs illustrated in Fig. 3. Assignment of the methyl signals is based on a study of the spectra of 24,28-$^2H_2$-24-methyl-5β-cholestane, and differs slightly from that reported previously [5]. In $CDCl_3$ all compounds give similar shifts for the C-26, 27 and 28 methyls depending on the chirality at C-24, with the exception of 14β-ergostane, which shows slight deshielding of all sidechain methyl signals. The C-27 methyl, which is the most sensitive to C-24 stereochemistry, is downfield (0·02ppm) in the 24α-series, a characteristic which is unaffected by substitution in rings A–C. In the 24β isomers the sequence Δ^5, Δ^7, $\Delta^{8(14)}$, Δ^{14} shows a gradual coalescence of the C-27/28 signals resulting from progressive slight deshielding of the C-28 methyl group. The C-21 methyl is sensitive to stereochemistry at C-24, being deshielded in the β series (0·01 ppm). However, this signal

Table 1. Methyl group chemical shifts of steroids containing achiral sidechains

$CDCl_3$	C-18*	C-19*	C-21†	C-26‡	C-27‡	C-29§
Cholesteryl acetate	0·673	1·014	0·908	0·858		—
Cholest-7-en-3β-yl acetate	0·536	0·811	0·919	0·865		—
Cholesta-5,*E*-22-dien-3β-ol	0·693	1·011	1·008	0·860		—
Cholesta-5,*Z*-22-dien-3β-ol	0·722	1·020	0·956	0·903	0·890	—
Fucosteryl acetate	0·686	1·021	0·985	0·980		1·576
28-Isofucosteryl acetate	0·680	1·020	0·945	0·975		1·590

* Singlet; † doublet (J 6·5 Hz); ‡ doublet ($J \sim$ 6·6 Hz); § doublet ($J \sim$ 6·6 Hz).

Table 2. Methyl group chemical shifts of 24-methyl steroids

$CDCl_3$	C-24 config.	C-18*	C-19*	C-21†	C-26‡	C-27‡	C-28‡
5α-Campestane	R/α	0·644	0·773	0·891	0·848	0·801	0·768
5β-Campestane	R/α	0·641	0·911	0·891	0·850	0·799	0·769
24,28-$^2H_2$-5β-Campestane	R/α	0·641	0·913	0·892	0·847	0·791	—
Campesterol	R/α	0·680	1·007	0·911	0·850	0·802	0·773
Campesteryl acetate	R/α	0·676	1·017	0·909	0·849	0·800	0·770
Campest-7-en-3β-ol	R/α	0·537	0·795	0·915	0·851	0·801	0·775
5α-Ergostane	S/β	0·641	0·773	0·900	0·850	0·777	0·770
5β-Ergostane	S/β	0·639	0·911	0·900	0·848	0·776	0·769
24,28-$^2H_2$-5β-Ergostane	S/β	0·641	0·913	0·900	0·847	0·778	—
14β-Ergostane	S/β	0·971	0·749	0·838	0·853	0·785	0·778
Dihydrobrassicasterol	S/β	0·678	1·009	0·919	0·852	0·783	0·775
Dihydrobrassicasteryl acetate	S/β	0·676	1·017	0·913	0·849	0·775	0·771
Ergost-7-en-3β-yl acetate	S/β	0·530	0·805	0·919	0·850	0·778	0·773
Ergost-8(14)-en-3β-yl acetate	S/β	0·834	0·702	0·929	0·850	0·777	0·777
Ergost-14-en-3β-yl acetate	S/β	0·895	0·839	0·911	0·852	0·783	0·783
C_6D_6							
5α-Campestane	R/α	0·689	0·799	1·018	0·915	0·870	0·870
5β-Campestane	R/α	0·678	0·968	1·033	0·927	0·881	0·881
24,28-$^2H_2$-5β-Campestane	R/α	0·680	0·968	1·030	0·927	0·878	—
Campesterol	R/α	0·677	0·955	1·014	0·915	0·871	0·871
Campesteryl acetate	R/α	0·662	0·923	1·022	0·922	0·878	0·878
Campest-7-en-3β-ol	R/α	0·616	0·756	1·018	0·919	0·875	0·875
5α-Ergostane	S/β	0·689	0·800	1·024	0·920	0·858	0·871
5β-Ergostane	S/β	0·680	0·971	1·033	0·931	0·870	0·883
24,28-$^2H_2$-5β-Ergostane	S/β	0·680	0·968	1·030	0·927	0·869	—
14β-Ergostane	S/β	1·095	0·765	0·972	0·906	0·845	0·876
Dihydrobrassicasterol	S/β	0·676	0·962	1·021	0·927	0·864	0·876
Dihydrobrassicasteryl acetate	S/β	0·662	0·923	1·022	0·922	0·865	0·878
Ergost-7-en-3β-yl acetate	S/β	0·592	0·733	1·011	0·922	0·859	0·871
Ergost-8(14)-en-3β-yl acetate	S/β	0·905	0·630	1·024	0·894	0·827	0·844
Ergost-14-en-3β-yl acetate	S/β	0·946	0·668	1·014	0·907	0·841	0·859

* Singlet; † doublet (J 6·5 Hz); ‡ doublet (J ~6·8 Hz).

is also influenced by substituents in the ring system and is deshielded (probably because of slight changes in conformation of the ring system) on introduction of double bonds into rings B and C.

The effect of an aromatic solvent (C_6D_6) on the diagnostic chemical shifts is somewhat different. Once again the terminal methyls are non-equivalent and the C-27 methyl signal is deshielded in the α-series, though less markedly than in $CDCl_3$ (0·012ppm). Substitution in rings A and B has no significant effect on the sidechain

Table 3. Methyl group chemical shifts of 24-ethyl steroids

$CDCl_3$	C-24 config.	C-18*	C-19*	C-21†	C-26‡	C-27‡	C-29§
5α-Stigmastane	R/α	0·645	0·773	0·904	0·832	0·808	0·841
5α-Stigmastanol	R/α	0·646	0·800	0·901	0·829	0·807	0·837
Sitosterol	R/α	0·680	1·007	0·919	0·833	0·813	0·842
Sitosteryl acetate	R/α	0·676	1·017	0·918	0·831	0·809	0·841
Stigmast-7-en-3β-ol	R/α	0·537	0·800	0·928	0·837	0·815	0·846
5α-Poriferastane	S/β	0·644	0·773	0·905	0·826	0·804	0·848
Clionasteryl acetate	S/β	0·673	1·013	0·920	0·825	0·805	0·845
Aplystanol	¶	0·648	0·800	0·890	0·805‖	0·793‖	0·854
Aplysterol	¶	0·678	1·005	0·904	0·807‖	0·792‖	0·855
C_6D_6							
5α-Stigmastane	R/α	0·695	0·802	1·038	0·917	0·896	0·927
5α-Stigmastanol	R/α	0·667	0·726	1·036	0·919	0·896	0·925
Sitosterol	R/α	0·678	0·955	1·034	0·918	0·896	0·927
Sitosteryl acetate	R/α	0·665	0·925	1·042	0·925	0·903	0·935
5α Poriferastane	S/β	0·693	0·802	1·047	0·915	0·901	0·940
Aplystanol	¶	0·674	0·715	1·025	0·910‖	0·885‖	0·906
Aplysterol	¶	0·678	0·952	1·021	0·910‖	0·883‖	0·906

* Singlet; † doublet (J 6·5 Hz); ‡ doublet (J 6·8 Hz); § triplet (J 7·2 Hz); ¶ 24*R*, 25*S* configuration; ‖ C-26/28 assignment uncertain.

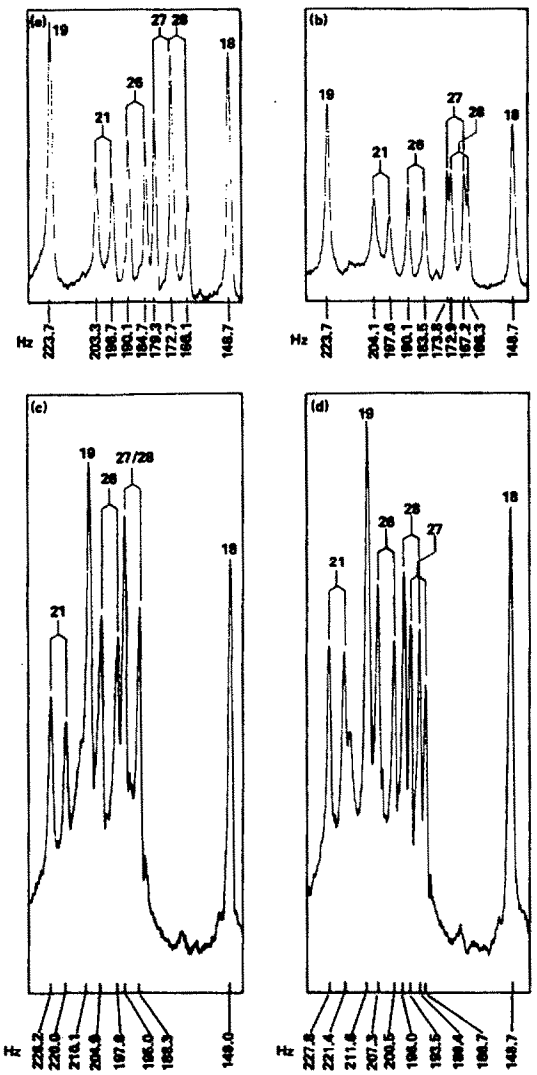

Fig. 3. 220 MHz NMR spectra of (a) campesteryl acetate, (24R/α) ($CDCl_3$); (b) dihydrobrassicasteryl acetate, (24S/β) ($CDCl_3$); [250 Hz sweep-width] (c) campesterol, (24R/α) (C_6D_6); (d) dihydrobrassicasterol (24S/β) (C_6D_6). [500 Hz sweep-width].

methyl signals. However, in the 24β-series ring C and D double bonds produce a general deshielding of these signals and a change in pattern. Slight perturbations are also produced by epimerisation at C-14 and, rather surprisingly, at C-5. The C-21 methyl chemical shifts are again at slightly lower field in the 24β epimers but are not significantly influenced by ring A/B substituents in this solvent.

In this series the differences between spectra of epimeric pairs are sufficient to permit semi-quantitative assessment of the proportion of the two epimers within a mixture. However, acquisition of spectra in both solvents is recommended to confirm such analyses.

24-ethylsteroids

The epimeric pairs of 24-ethylsteroids also show non-equivalence of the terminal *iso*-propyl methyl groups (Table 3), though in $CDCl_3$ the splitting is generally less

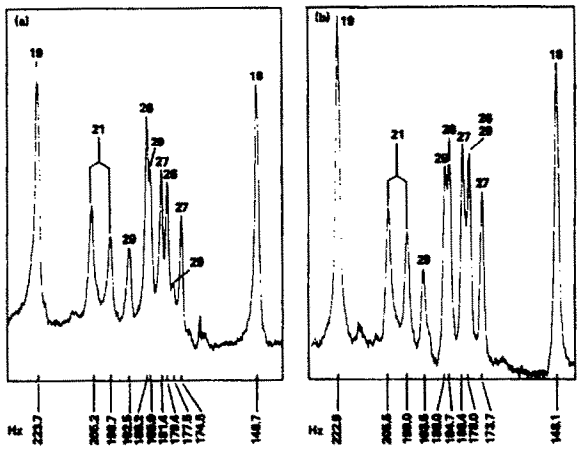

Fig. 4. 220 MHz NMR spectra of (a) sitosteryl acetate (24R/α) ($CDCl_3$); (b) clionasteryl acetate (24S/β) ($CDCl_3$). [250 Hz sweep-width].

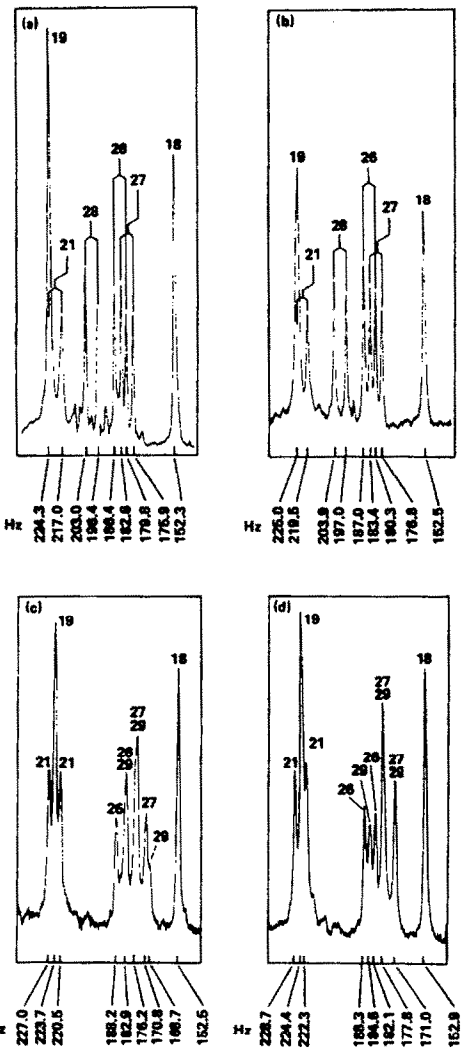

Fig. 5. 220 MHz NMR spectra of (a) (24S)-24-methylcholesta-5,*E*-22-dien-3β-yl acetate (24S/α); (b) brassicasteryl acetate (24R/β); (c) stigmasteryl acetate (24S/α); (d) poriferasteryl acetate (24R/β) (All in $CDCl_3$).

than in the 24-methyl series. This is consistent with the lower polarisability of an ethyl substituent relative to a methyl group [24]. The C-26 and C-27 methyl signals show slight chemical shift differences between epimers, attributable to the long-range influence of the ring system. However, the major diagnostic feature is the chemical shift of the C-29 methyl triplet which is deshielded (~0·006ppm) in the β-series, causing a marked difference in the pattern of signals within spectra of the two series (Fig. 4). Substituents in rings A/B have no effect on this pattern. While the influence of ring double bonds on the C-21 methyl is again significant, the chemical shift of this group is not affected by C-24 stereochemistry in $CDCl_3$. However, in C_6D_6 the C-21 and C-29 methyls show enhanced differences between C-24 epimers, both signals again appearing at lower field in the β isomers. Thus the aromatic solvent gives a clearer indication of stereochemistry in this series.

Assessment of the composition of a mixture of epimers is difficult in this group. However, careful analysis of the C-29 triplet signals and comparison of spectra with those of epimer mixtures of known composition should enable such determinations to be made with reasonable accuracy.

The spectra of aplystanol and aplysterol (24*R*, 25*S*-24,26-dimethyl-cholest-5-en-3β-ol) [25] are included in Table 3 for comparison. The chemical shifts of the sidechain methyl groups in these isomers are clearly different from those of the isomeric 24-ethyl steroids, reflecting the interaction of two adjacent chiral centres in the sidechain.

Δ^{22}-24-*alkylsteroids*

In the presence of a *trans* Δ^{22} double bond the non-equivalence of the C-26/7 methyls in 24-methylsteroids is less than that observed in the sidechain-saturated analogues in both solvents (Table 4). In $CDCl_3$ the C-21 methyl signal is again deshielded in the β-series, though the C-28 methyl is not sensitive to stereochemistry, [assignment of the C-28 methyl signal follows from collapse of the doublet at 0·915δ in ergosteryl acetate on irradiation at 1·9δ (allylic proton position)]. No direct comparisons are yet available for C_6D_6. It would appear that, in this series, assignment of C-24 stereochemistry should be confirmed by reduction of the Δ^{22} double bond since, as shown above, the spectral differences between epimers in the saturated series are rather more diagnostic.

The Δ^{22}-24 ethyl steroids which have been examined exhibit, in $CDCl_3$, diagnostic deshielding of the C-21 and 29 methyl signals in the β series while the *iso*-propyl group is insensitive to C-24 chirality (Fig. 5). In C_6D_6 slight differences are observed in all sidechain methyl signals, relative deshielding of the C-21 and C-29 signals in the 24β epimer being larger than in $CDCl_3$. Therefore, the aromatic solvent is marginally preferable for stereochemical analysis of this group.

EXPERIMENTAL

Source of sterols. In most cases the NMR spectra were determined on the steryl acetate, since this is the most convenient derivative to use during the purification of naturally occurring sterols by $AgNO_3$–Si gel chromatography. Cholesteryl acetate (cholest-5-en-3β-yl acetate) was supplied by B.D.H. Ltd. Cholest-7-en-3β-yl acetate was isolated [26] from *Asterias rubens*. Cholesta-5,*E*-22-dien-3β-ol and cholesta-5,*Z*-22-dien-3β-ol

Table 4. Methyl group chemical shifts of Δ^{22}-24 alkyl steroids

$CDCl_3$	C-24 config.	C-18*	C-19*	C-21†	C-26‡	C-27‡	C-28‡	C-29§
(24*S*)-24-Methylcholesta-5,*E*-22-dien-3β-yl acetate	S/α	0·693	1·018	1·001	0·833	0·816	0·907	—
(24*S*)-24-Methylcholesta-7,*E*-22-dien-3β-yl acetate	S/α	0·542	0·813	1·007	0·836	0·820	0·911	—
Brassicasterol	R/β	0·694	1·010	1·001	0·834	0·818	0·909	—
Brassicasteryl acetate	R/β	0·693	1·023	1·003	0·832	0·815	0·909	—
(24*R*)-24-Methylcholesta-7 *E*-22-dien-3β-yl acetate	R/β	0·544	0·813	1·015	0·832	0·817	0·910	—
Ergosteryl acetate	R/β	0·626	0·950	1·034	0·836	0·821	0·915	—
Stigmast-22-ene	S/α	0·662	0·775	1·003	0·841	0·791	—	0·797
Stigmast-22-ene-3β-ol	S/α	0·664	0·801	1·000	0·840	0·787	—	0·795
Stigmasteryl acetate	S/α	0·693	1·017	1·017	0·842	0·791	—	0·799
Spinasteryl acetate	S/α	0·544	0·819	1·030	0·853	0·805	—	0·808
Poriferasteryl acetate	R/β	0·695	1·020	1·025	0·842	0·791	—	0·808
Chondrillasteryl acetate	R/β	0·554	0·821	1·035	0·850	0·800	—	0·820
C_6D_6								
(24*S*)-24-methylcholesta-7,*E*-22-dien-3β-yl acetate	S/α	0·599	0·738	1·017	0·923	0·918	1·009	—
Brassicasterol	R/β	0·678	0·955	1·112	0·917	0·917	1·006	—
Ergosteryl acetate	R/β	0·648	0·914	1·099	0·917	0·910	1·003	—
Stigmast-22-ene	S/α	0·697	0·799	1·128	0·955	0·905	—	0·937
Stigmast-22-ene-3β-ol	S/α	0·674	0·712	1·126	0·955	0·905	—	0·937
Stigmasteryl acetate	S/α	0·661	0·924	1·112	0·949	0·900	—	0·933
Poriferasteryl acetate	R/β	0·664	0·927	1·121	0·960	0·912	—	0·945

* Singlet; † doublet (J 6·5 Hz); ‡ doublet (J ~6·8 Hz); § triplet (J ~7·2 Hz).

were gifts from Dr. M. J. Thompson, Insect Physiology Laboratory, U.S. Department of Agriculture, Beltsville, Maryland, U.S.A. Fucosteryl acetate (stigmasta-5,*E*-24(28)-diene-3β-yl acetate) was obtained from *Fucus spiralis* and 28-isofucosteryl acetate (stigmasta-5,*Z*-24(28)-dien-3β-yl acetate) was isolated from *Enteromorpha intestinalis* [27]. The hydrocarbons were synthesised by standard methods from the corresponding sterols. 14β-Ergostane [14β-(24*S*)-24-methylcholestane] was prepared from ergosterol via 7-keto-ergost-8(14)-en-3β-yl acetate [28]. Campesterol [(24*R*)-24-methylcholest-5-en-3β-ol] was purchased from Applied Science Laboratories Inc. Dihydrobrassicasterol [(24*S*)-24-methylcholest-5-en-3β-ol], brassicasterol [(24*R*)-24-methylcholesta-5,*E*-22-dien-3β-ol], their acetates and campest-7-en-3β-ol [(24*R*)-24-methylcholest-7-en-3β-ol] were gifts from Dr. H. W. Kircher, Department of Agricultural Biochemistry, University of Arizona, Tucson, Arizona, U.S.A. (24*S*)-24-Methylcholesta-5,*E*-22-dien-3β-yl acetate was isolated from *Orthosiphon nigra* [17]. (24*R*)-24-Methylcholesta-7,*E*-22-dien-3β-yl acetate was synthesised by reduction of ergosteryl acetate and (24*S*)-24-methylcholesta-7,*E*-22-dien-3β-yl acetate was isolated from *A. rubens* [26]. (24*S*)-24-methylcholest-8(14)-en-3β-yl acetate and (24*S*)-24-methylcholest-14-en-3β-yl acetate were synthesised from ergosterol. Sitosteryl acetate [(24*R*)-24-ethylcholest-5-en-3β-yl acetate] was isolated from *Lupinus luteus* [30]. Clionasteryl acetate [(24*S*)-24-ethylcholest-5-en-3β-yl acetate] was a gift from Dr. G. W. Patterson, Department of Botany, College of Agriculture, University of Maryland, Maryland, U.S.A. Aplystanol [24*R*,25*S*-24,26-dimethylcholestan-3β-ol] and aplysterol [24*R*,25*S*-24,26-dimethylcholest-5-en-3β-ol] were gifts from Dr. L. Minale, Laboratorie per la Chimica e Fisica del C.N.R., Arco Felica (NA), Italy. Stigmasteryl acetate [(24*S*)-24-ethylcholesta-5,*E*-22-dien-3β-yl acetate] was purchased from Steraloids. Poriferasteryl acetate [(24*R*)-24-ethylcholesta-5,*E*-22-dien-3β-yl acetate] was isolated from *Ochromonas malhamensis* [15]. Spinasteryl acetate [(24*S*)-24-ethylcholesta-7,*E*-22-dien-3β-yl acetate] was isolated from *Spinacea oleracea* [31]. Chondrillasteryl acetate [(24*R*)-24-ethylcholesta-7,*E*-22-dien-3β-yl acetate] was isolated from *Scenedesmus obliquus*.

Determination of spectra. The 220MHz NMR spectra were measured on Varian HR-220 spectrometers at the Physical Chemical Measurements Unit, Harwell, U.K., and at Central Laboratory, T.N.O., Delft, Holland. The compounds were dissolved in $CDCl_3$ or C_6D_6 (0·8–5% soln) using TMS as internal standard. Sweep width was either 250 or 500 Hz and the probe temp. 19–22° (P.C.M.U.) or 25–29° (T.N.O.). Reproducibility of chemical shifts is within 0·5 Hz (±0·002δ) on each spectrometer.* Comparison of values for compounds measured on both spectrometers indicates a reproducibility of ca ±0·005δ. The contribution of slightly different probe temperatures to this larger variation is probably significant.

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* We have found that differences in the spectra of isomeric pairs of sterols can be most readily seen if the spectra are superimposed and viewed with illumination from beneath.

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