Sterols of Scallop. III. Characterization of Some C-24 Epimeric Sterols by High Resolution (220 MHz) Nuclear Magnetic Resonance Spectroscopy[1]

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ABSTRACT

Alkyl sterols epimeric at C-24 isolated from the Atlantic scallop, *Placopecten magellanicus*, were analyzed by high resolution (220 MHz) nuclear magnetic resonance spectrometry, and their spectra compared with authentic samples. This technique was used to assign absolute stereochemistry in epimeric mixtures of 24 R and S 24-methylcholest-5-en-3β-ol, (22E)-24-methylcholesta-5,22-dien-3β-ol and 24-ethylcholest-5-en-3β-ol. It also allowed a semiquantitative estimate of the R/S isomers present in the mixture.

INTRODUCTION

Marine invertebrates have been shown to contain complex sterol mixtures, and although many new sterols were discovered (1) the analytical procedures available at that time were unable to resolve these complex mixtures completely. More recent investigations into the sterols of marine invertebrates have been reviewed (2). We recently re-examined the sterol mixture of the scallop *Placopecten magellanicus* and isolated several minor sterols as well as several epimeric 24-alkycholesterols (3). High resolution ^1H NMR has been used to evaluate the stereochemistry of sterols epimeric at C-24 (4-6), and we have reported on the utility of ^{13}C NMR for determining stereochemistry at C-24 (7). However, the smaller amounts of samples required and the ready availability of ^1H NMR prompted us to use this method, and to compare the findings with those of the ^{13}C NMR study.

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EXPERIMENTAL

The NMR spectra were recorded at 220 MHz on a Varian spectrometer, using $CDCl_3$ solvent and TMS as internal standard. Spectra were recorded at a sweep width of 100 Hz. Peak positions in the spectra are given in Hz and were converted to ppm (Tables I and II) by dividing the values by 220 for the 220 MHz spectra. Epimeric ratios were estimated by planimetry of peak heights.

24-Alkyl sterols were isolated from scallop in a previous study (3). One sample of campe-

TABLE I

Methyl Group Chemical Shifts of 24-Methyl and 24-Methyl-Δ^{22}-Sterols in $CDCl_3$

Sterol	C-24 Config.	C-18[a]	C-19[a]	C-21[b]	C-26[b]	C-27[b]	C-28[b]
Mixture *1a* and *1b* (scallop)	S/β R/α	0.679[c]	1.007	0.919 0.911	0.856 0.852	0.783 0.704	0.775
Mixture *1a* and *1b* (synthetic)	S/β R/α	0.682	1.012	0.923 0.915	0.860	0.785 0.814	0.780
Mixture *1a* and *1b* (nutritional biochem.)	S/β R/α	0.685	1.013	0.923 0.916	0.856	0.787 0.808	0.778
Campesterol (appl. sci.) *1b*	R/α	0.687	1.012	0.915	0.857	0.808	0.779
(22E,24ξ)-24-Methylcholesta-5,22-dien-3β-ol *3a* and *3b* (scallop)	S/α R/β	0.698	1.012	1.007 1.015	0.841	0.823	0.915 0.934
(22E,24S)-24-Methylcholesta-5,22-dien-3β-ol *3a* (synthetic)	S/α	0.700	1.013	1.006	0.841	0.823	0.915
22E,24S)-24-Methylcholesta-5,22-dien-3β-ol *3a* (diatom)	S/α	0.699	1.015	1.009	0.842	0.824	0.916

[a]Singlet.

[b]Doublet.

[c]All values given in ppm.

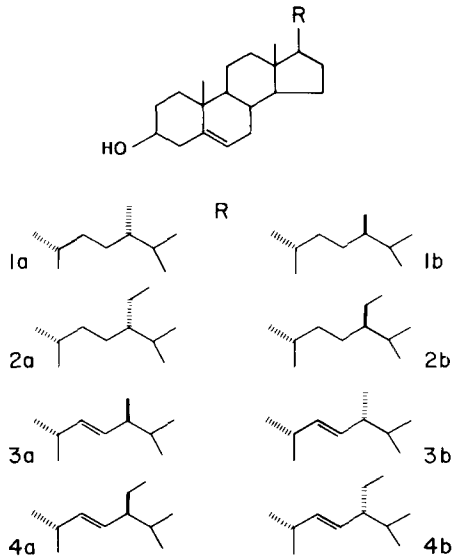
TABLE II

Methyl Group Chemical Shifts of 24-Ethyl and 24-Ethyl-Δ^{22}-Sterols in $CDCl_3$

Sterol	C-24 Config.	C-18	C-19	C-21	C-26	C-27	C-28
Clionasterol *2a*	S/β	0.683[a]	1.011	0.928	0.834	0.814	0.855
Sitosterol *2b*	R/α	0.684	1.011	0.925	0.839	0.815	0.842
Mixture of *2a* and *2b* (scallop)	S/β R/α	0.683	1.006	0.925	0.839	0.816	0.848
Poriferasterol *4b*	R/β	0.699	1.009	1.025	0.845	0.793	0.880 0.812
Mixture of *4a* and *4b* (scallop	S/α R/β	0.701	1.014	1.028	0.848	0.795	0.814
Stigmasterol (24S) *4a*	S/α	0.703	1.012	1.026	0.849	0.799	0.808

[a]All values given in ppm.

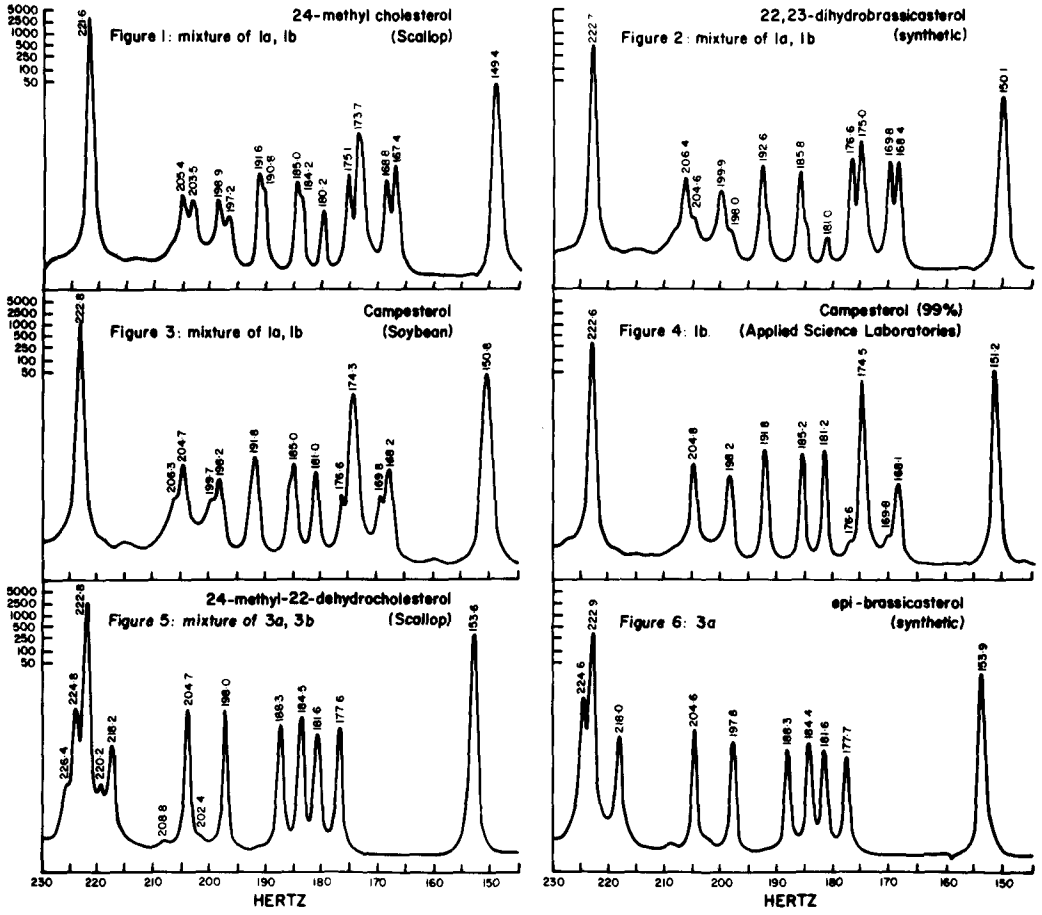
sterol *1b* was purchased from Applied Science Laboratories, State College, PA, while a second sample of "campesterol" *1b* from soybean was obtained from Nutritional Biochemicals, Cleveland, OH, and contained approximately one-third 22, 23-dihydrobrassicasterol *1a*. Clionsterol (24S)-24-ethylcholest-5-en-3β-ol *2a* was isolated from *Nitella* (8) while sitosterol (24R)-24-ethylcholest-5-en-3β-ol *2b* was isolated from soybean. 22-Dehydrocampesterol (22E, 24R)-24-methylcholesta-5,22-dien-3β-ol, *3a*, was isolated from the diatom *Phaeodactylum tricornutum*. Dr. M.J. Thompson (USDA, Beltsville, MD) provided synthetic samples of *3a* (epibrassicasterol) and *1a* (dihydrobrassicasterol), and the latter was shown to contain approximately one-third campesterol *1b*. Stigmasterol (22E,24S)-24-ethycholesta-5,22-dien-3β-ol), *4a*, was purchased from Applied Science Labs, while poriferasterol, the 24R epimer was obtained from *Spirogyra*, a fresh water alga.



RESULTS AND DISCUSSION

(24R)-24-Methylcholest-5-en-3β-ol *1b* and (24S)-24-Methylcholest-5-en-3β-ol *1a*

The methyl group chemical shifts of four samples of 24-methylcholesterol are listed in Table I, and the spectra are illustrated in Figures 1-4; these are scallop sterol (Fig. 1), a synthetic sample of "22,23-dihydrobrassicasterol" (Fig. 2), and campesterol samples from soybean (Nutritional Biochemicals) and Applied Science. Assignments of the methyl group of campesterol (Applied Science, Table I) agree well with published data (4-6), but comparison of the spectrum of the scallop sample shows certain differences (Table I). In the scallop sterol spectrum, there are two pairs of doublets at 0.911 and 0.919 ppm (Table I) assigned to the C-21 methyl resonance of the 24R and 24S epimers, respectively. This difference of ∿2 Hz is also found in the spectra of the other epimeric mixtures of 24-methylcholesterol examined. There are two pairs of doublets at 0.783 and 0.804 ppm assigned to C-27 methyl resonances for the 24S and 24R epimers. The chemical shift difference of 0.02 ppm (4.4 Hz) is constant for the epimeric mixtures examined, and agrees with the observation by Rubinstein et al. (5) that the C-27 methyl resonance is most sensitive to C-24 stereochemistry and is downfield in 24α sterols (e.g., *1b*); it is therefore of diagnostic value in assigning C-24 stereochemistry in saturated 24-methyl sterols. Estimation of peak heights of the C-21 methyls established that the scallop sterol is an approximately equal mixture of the 24R and 24S epimers; the synthetic sample (Fig. 2) is a mixture containing two-thirds *1a*, while the soybean sample (Fig. 3) contains approximately equal amounts of *1a* and *1b*; a small amount of the 24S epimer was detected in campesterol from Applied Science.



FIGS. 1-6. 220 Hz spectra of sterols; sweep width 100 Hz.

(22E,24R)-24-Methylcholesta-5,22-dien-3β-ol *3b* (Brassicasterol), and (22E,24S)-24-Methylcholesta-5, 22-dien-3β-ol *3a* (22-dehydrocampesterol)

Three samples were examined: a diastereoisomeric mixture from the scallop (Fig. 5), a sample of the 24S isomer, 22-dehydrocampesterol (Fig. 7) from the diatom *P. tricornutum*, and a synthetic sample, also with 24S stereochemistry (Fig. 6). The methyl group chemical shifts are summarized in Table I. Inspection of the spectra shows that the 24R and S epimers can be distinguished by the differences in the chemical shift of the C-21 methyl, which is at lower field in the 24R (β) series. The C-28 methyl group is less sensitivie to stereochemistry.

The sensitivity of the C-21 methyl group to stereochemistry in ^{1}H NMR offers an additional diagnostic tool since ^{13}C NMR (7) has shown that the most useful signals for differentiating these epimers are the resonances at C-28 and C-16. Chemical shift differences for the C-21 and C-28 resonances using 100 MHz ^{1}H NMR were also used to assign stereochemistry at C-24 (9) in the diatom *P. tricornutum*.

Estimates that the scallop sample contains about two-thirds 24S epimer, 3a(∿65%) by integration of the C-21 methyl resonance, though not as precise, are in good agreement with values from ^{13}C NMR (7). These new data confirm the earlier suggestion that (22E,24ξ)-24-methylcholesta-5,22-dien-3β-ol from scallop might be a mixture of the C-24 epimers from which brassicasterol was isolated (10). Synthetic *3a* (Fig. 6) is included for comparison and is very similar to the diatom sterol (Fig. 7) whose assignment (9) as 24S is confirmed. The Japanese scallop *Patinopecten yessoensis* has been shown to contain *3a* (11), but these workers did not mention whether it occurred along with lesser amounts of *3b*, as in *P. magellanicus*.

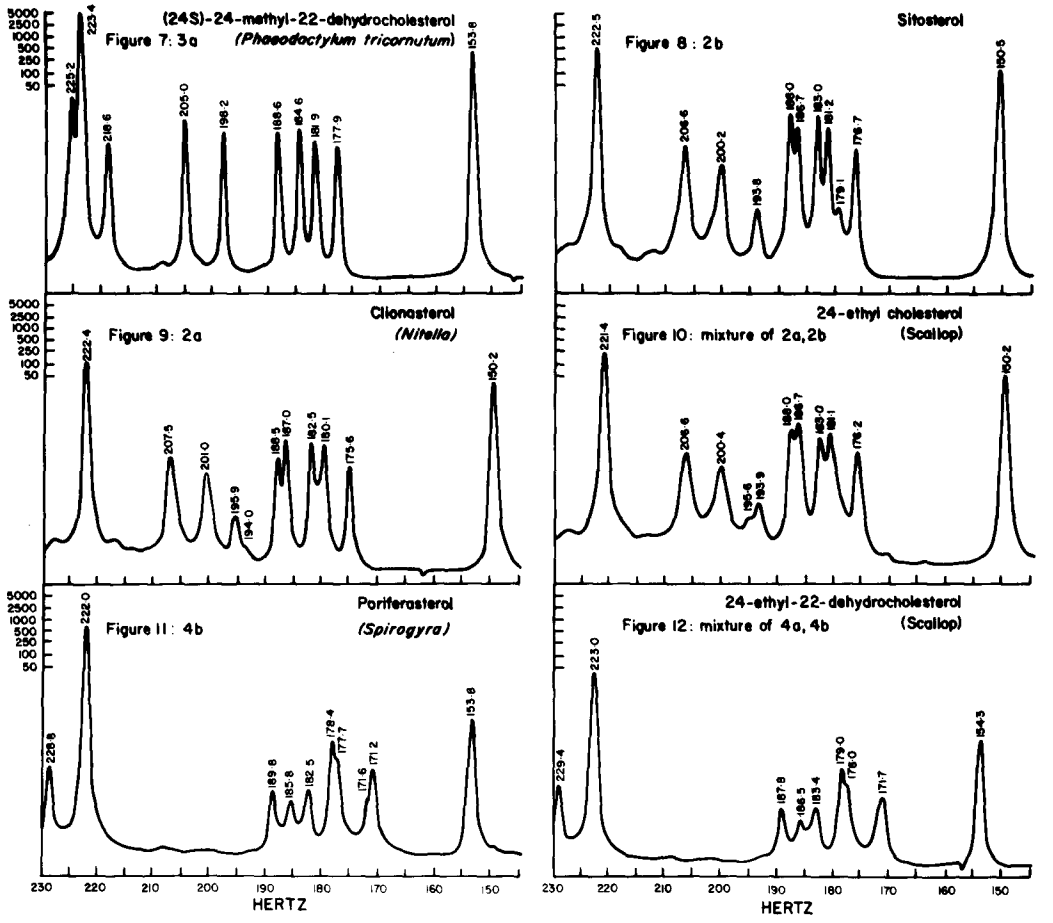
(24R)-24-Ethylcholest-5-en-3β-ol *2b* and (24S)-24-Ethylcholest-5-en-3β-ol *2a*

Three samples were examined: sitosterol *2b* from soybean (Fig. 8), clionasterol *2a* (Fig. 9) isolated as the acetate from Nitella (8), and a mixture isolated from scallop (Fig. 10). Previous studies (5,6) have realized the difficulty of differentiating C-24 diastereoisomers of C_{29} sterols, although differentiation by ^{13}C NMR has already been pointed out (7). The C-29 methyl resonance of the 24S epimer 2a is more deshielded than that in the 24R isomer *2b*, and offers the only major difference. It is, however, necessary to obtain spectra of both diastereoisomers; and comparison shows that the scallop sterol is clearly a mixture with the 24R epimer the major sterol (∿65%). While precise determinations of epimeric ratios are not possible by ^{1}H NMR as with ^{13} C NMR (67%R:33%S) (7), the former provided a quite good estimation of the relative amounts in this instance. When pure samples are available, the acetate of *2b* m.p. 122C can be distinguished from 2a acetate m.p. 143 C (4).

(22E,24R)-24-Ethylcholesta-5,22-dien-3β-ol-*4b* and (22E,24S)-24-Ethylcholesta-5,22-dien-3β-ol-*4a*

Three samples were examined: pure 24R (poriferasterol, Fig. 11), 24S (stigmasterol, Fig. 13), and an epimeric mixture from scallop (Fig. 12). Methyl group chemical resonances are recorded in Table II. Comparison of these spectra shows that the scallop sterol contains predominantly the 24R epimer *4b*. As for mixtures of compounds *2a* and *2b*, ^{1}H NMR does not easily differentiate between the R and S epimers in a mixture of *4a* and *4b*, but estimation is still possible with ^{13}C NMR (7).

In summary, 220 MHz ^{1}H NMR was used to assign configuration at C-24 of mixtures of 24 alkyl sterols isolated from the scallop, by comparison with authentic samples, and to give a qualitative estimate of the R/S ratios.



FIGS. 7-12. 220 Hz spectra of sterols; sweep width 100 Hz.

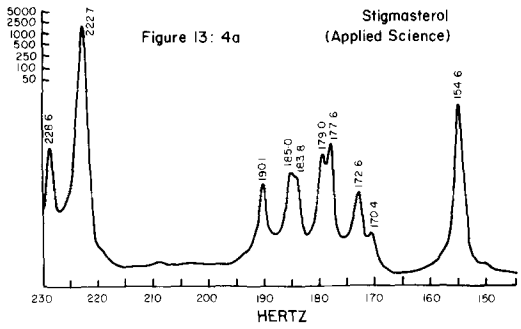


FIG. 13. 220 Hz spectra of sterols; sweep width 100 Hz.

Previous studies (4-6,12) have also demonstrated the functional utility of this method, and have noted its limitations. It has been pointed out that in higher plants C_{28} and C_{29} sterols (24α) with saturated side chains are more common than those with 22-double bonds, whereas in algae the reverse is true (13). In scallop, which apparently obtains sterols from many sources, 24α sterols (e.g., *1b,2b*) predominate when the side chain is saturated, while in the 22-dehydrosterols, scallop contains mixtures of both 24α (e.g., *3a*) and 24β (e.g., *4b*) epimers (Tables I-II and Figs. 1-3).

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