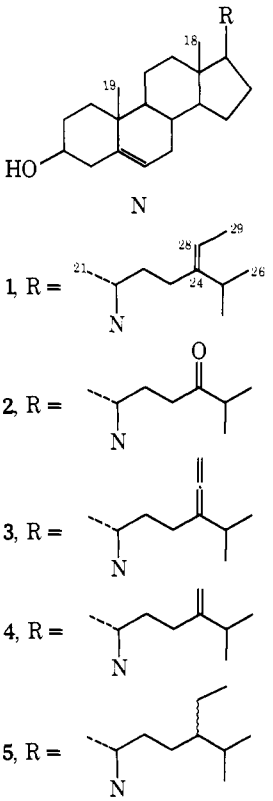
24-Ethyl-$\Delta^{5,24(28),28}$-cholestatrien-3β-ol—a Naturally Occurring Allenic Marine Sterol[1]

Sir:

Marine sterols are characterized by unusual structural features in their side chains[2] such as cyclopropane,[3,4] cyclopropene,[5] and acetylene[6] groupings. We now report the first encounter of a naturally occurring sterol possessing an allenic side chain. Several years ago Erdman and Scheuer[7] examined the sterol composition of *Callyspongia diffusa* and identified nine known sterols, the principal among them being isofucosterol (**1**). Utilizing our recently described[8] separation and analysis methods for detecting trace sterols we reinvestigated the sterol mixture of this sponge and isolated, in addition to the described sterols, 24-ketocholesterol (**2**)—a possible degradation product of isofucosterol (**1**)—and a new sterol (mp 113–114 °C) shown to be 24-ethyl-$\Delta^{5,24(28),28}$-cholestatrien-3β-ol (**3**).



The mass spectrum of **3** exhibits a molecular ion of 410 (410.3516, calcd for $C_{29}H_{46}O$ 410.35485) which indicates three degrees of unsaturation, assuming an intact steroid nucleus. The peaks at *m/e* 271, 281, 299, and 314 are typical[9] for an unsaturated side chain such as 24-methylenecholesterol (**4**). Together with the nuclear fragment ions at *m/e* 213, 229, and 255, they clearly indicate that the second unsaturation must also be in the side chain and that the third one is in the nucleus at Δ^5 or Δ^7. The 360-MHz ^{1}H NMR spectrum shows only five methyl group signals (C-18, 0.673; C-19, 1.003; C-21, 0.930 (d); C-26,27, 1.010 ppm (d)),[10] thus ruling out methyl groups other than those present in cholesterol. A pseudoquartet (2 H, 3 Hz) at 4.68 ppm is typical[11] for a terminal methylene group, and a double resonance experiment showed that these protons couple with the C-25 hydrogen. The ^{13}C NMR spectrum (Table I) displays, beside the signals corresponding to those of cholesterol,[12] three distinct signals at 204.3, 110.1, and 76.5 ppm (triplet in off-resonance experiment), which clearly indicate a terminal allenic structure.[13] This is confirmed by the characteristic allene infrared absorption at 1950 cm^{-1}. The position of the allene system can be derived from the mass spectrum; a typical[14] McLafferty rearrangement to the central allene carbon yields an *m/e* 314 fragment which requires the presence of a $\Delta^{24(28),28}$-diene structure. The 3β-hydroxy-Δ^5-steroid nucleus is confirmed by the ^{1}H and ^{13}C NMR spectra (Table I). Thus all spectral data of this new marine sterol are in perfect agreement with structure **3**. Final proof came from the catalytic hydrogenation of **3** with PtO_2, which led to a mixture of C-24 epimeric sitosterols (**5**).

Table I. ^{13}C Chemical Shifts ($CDCl_3$) of 24-Ethyl-$\Delta^{5,24(28),28}$ cholestatrien-3β-ol (**3**)

carbon	δ_{Me_4Si}	carbon	δ_{Me_4Si}	carbon	δ_{Me_4Si}
28	204.82	4	42.37	25	30.43
5	140.80	13	42.29	16	28.14
6	121.62	12	39.83	23	26.92
24	110.07	1	37.30	15	24.28
29	76.53	10	36.51	26, 27	21.65
3	71.72	20	35.49	11	21.11
14	56.79	22	34.05	19	19.38
17	56.16	7, 8	31.94	21	18.69
9	50.19	2	31.64	18	11.87

This first isolation of a steroidal allene is of obvious biogenetic interest since Minale and collaborators[15] recently showed that isofucosterol (**1**) is a biosynthetic precursor of the steroidal cyclopropene calysterol. It is conceivable that isofucosterol, which is a major sterol of *Callyspongia diffusa*,[7] similarly is a precursor of 24-ethyl-$\Delta^{5,24(28),28}$-cholestatrien-3β-ol (**3**), but labeling experiments would have to be performed to establish this point. Even more interesting is the fact that our allene **3** had actually been synthesized by Ikekawa et al.[16] in the course of a synthetic program looking for possible inhibitors of sterol biosynthesis. The synthetic allene **3** was found by them to be a specific inhibitor in the silkworm *Bombyx mori* in the transformation of sitosterol to fucosterol. It remains to be seen whether our isolation of this allene in nature indicates a specific biological role and what this role is in the marine environment.

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References and Notes

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Chiral Aggregation Phenomena. 1. Acid Dependent Chiral Recognition in a Monolayer

Sir:

We present here a preliminary report of surface tension measurements and force-area curves which demonstrate a clear acid dependence between molecular packing in enantiomeric and racemic *N*-α-methylbenzylstearamide (I) monolayers. The observation may be of importance to the study of biomembranes. To the best of our knowledge it is also unprecedented and is probably quite general.

Figure 1 shows that films of I spread on aqueous sulfuric acid solutions are surface active (by du Noüy tensiometer), commencing at ~1 N acid for racemic amide and 4.5 N acid for pure (*S*)-(−) or (*R*)-(+) enantiomers. Figure 2 shows the same type of acid-dependent discrimination as manifested through force-area curves at different acidities.[1,2]

It is significant that chiral discrimination commences at rather large molecular areas (85–90 Å^2/molecule) where intermolecular packing is still quite loose. Figure 2 shows clearly that enantiomer–racemate discrimination becomes increasingly pronounced as the molecular area is reduced even though both films are monolayers and no sharp phase changes have occurred. The force required to pack racemic molecules is consistently higher, at a given molecular area, than is that for pure enantiomers. In harmony with this observation, Figure 1 shows that, at 60 Å^2/molecule, the racemic monolayer is more easily expanded as a function of increasing acid than are the enantiomeric monolayers.

Biomembranes are very thin multilayers composed largely of chiral amphiphilic molecules. The significance of our observation to membrane study comprises the following. (a) Chiral recognition probably can occur in multilayers even at molecular separations which are considerably larger than those encountered in lipid crystals. (b) Chiral recognition for oriented biological systems such as membranes may be quite pH dependent. (c) Monolayer studies of racemic lipids as models for optically active natural material are necessarily inconclusive. (d) Chiral discrimination between pure enantiomeric and racemic mixtures has previously been reported for several long-chain alcohols,[3-5] their esters,[5] and a polyamide.[6] Similar behavior of the amide reported here, despite its drastically different shape, implies that such discrimination is not a very subtle effect and is probably quite general.

In view of the small (30 μg) quantities of material used to establish the monolayer, and also the notorious sensitivity of monolayer studies to impurities, we stress the significance of the *absolute method* used here to confirm that the observed differences between films spread from enantiomers and those cast from the racemate are truly due to differences in intermolecular packing rather than to artifacts.[7] Enantiomers are perfect models for each other since all of their physical properties are identical except for their interactions with polarized light or with other chiral materials. Thus, the purity of enantiomeric monolayers is demonstrated when all physical properties, including their surface behavior, are the same within experimental error. When this criterion is met, their (enantiomeric) behavior with each other or their (diastereomeric) behavior with other chiral surfactants can be assigned rigorously to stereospecific intermolecular packing within the monolayer.

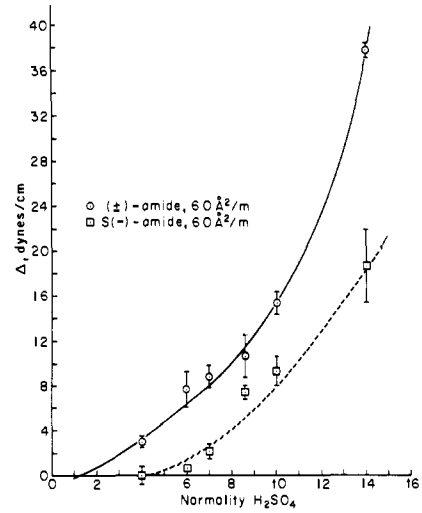

Figure 1. Surface tension lowering (Δ) by racemic and (*S*)-(−)-*N*-α-methylbenzylstearamide films at 25 °C and molecular area of 60 Å^2/molecule as a function of subphase acidity.

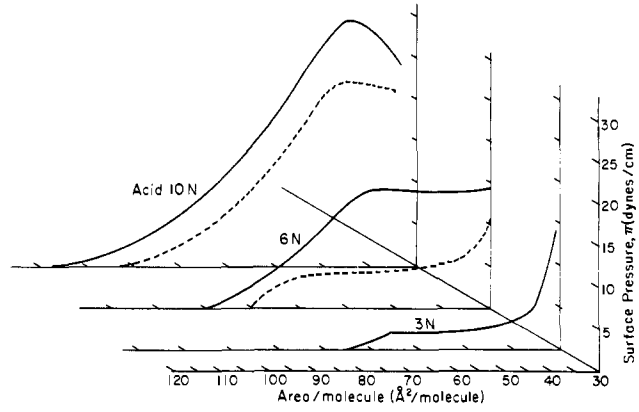

Figure 2. Force-area surves for compression of monolayers of racemic (−) and pure enantiomeric (*R* and *S*) (- - -) *N*-α-methylbenzylstearamide at 25 °C spread from hexane on 3, 6, and 10 N H_2SO_4.

The stearamides were prepared by aminolysis of highly purified methyl stearate with (*R*)-(+)- and (*S*)-(−)-α-methylbenzylamine. The resulting enantiomeric amides were purified to identical physical properties and were spread on aqueous solutions prepared from triply distilled water and distilled sulfuric acid using purified hexane as a spreading solvent. Racemic monolayers were produced both from racemic amide and by spreading a 1:1 solution of the pure (*R*)-(+) and (*S*)-(−) enantiomers. Results were identical. Correspondingly, all measurements with pure enantiomer were checked with both antipodes.

The film balance (designed by B.K.) was operated at 25 ± 0.2 °C at a normal compression rate of 20 Å^2 molecule^{-1} min^{-1}. Surface pressures could be measured to ±1.4%. Features of the force-area curves were reproducible to ±2% for the enantiomers and to ±5% for the racemate molecules as determined from the standard deviations of 22 points derived from 14 replica curves for the enantiomers and 10 curves for the racemate. Curves shown in Figure 1 are the composite averages for such samples. Surface tensions were measured with a Cenco du Noüy type tensiometer calibrated against

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