perience steric compression from the 14β-methyl group. It is to relieve the steric strain thereby induced that rotation of 180° proceeds yielding a skew conformer (**5b**). This right-handed conformer (**5b**) then permits completion of the reaction by elimination of the substituent at C-20 in a trans-reaction. The elimination and consequent migration of the 17β-H-atom to C-20 in turn invert C-20 which as a result of simultaneous inversion at C-17 produces the stable skew conformer (**3b**) of the completed sterol.[17] The presumed facility of the conformational change from **5a** to **5b** is in keeping with the work of van Tamelen and co-workers[18] who have found that the overall cyclization is not particularly sensitive to the nature of R which can vary between H and the full structure of the natural side chain.

Acknowledgment. This work was supported by National Institutes of Health Grants AM-12172 and GM-20526.

References and Notes

(1) The dominant sterols are those comprising ca. 95% of the sterol mixture and apparently are the functional ones acting primarily in membranes as discussed in detail by W. R. Nes, *Lipids*, **9**, 596 (1974).
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(9) I. Rubinstein, L. J. Goad, A. D. H. Clague, and L. H. Mulheirn, *Phytochemistry*, **15**, 195 (1976).
(10) Conformational isomerism about the 17(20)-bond of 20-ketosteroids, however, has been well investigated. For a key to the literature see W. R. Nes and T. E. Varkey, *J. Org. Chem.*, **41**, 1652 (1976).
(11) W. R. Nes, T. E. Varkey, D. R. Crump, and M. Gut, *J. Org. Chem.* **41**, 3429 (1976). The same isomerization was also observed in the conversion of (*Z*)-17(20)-dehydrocholesterol to 17α,20α-dihydroxycholesterol by osmylation and reduction. These results led to the expectation of those presently reported.
(12) This was prepared by the Grignard reaction with pregnenolone followed by acid-catalyzed dehydration. For details and a key to the configurational literature, see W. R. Nes et al.[11] ^{1}H NMR spectra were determined in $CDCl_3$ at 220 MHz. The spectrum of 20-isocholesterol was the same as that of a sample prepared by another route: Y. Letourneux, Ph.D. Thesis, Universite de Paris-Sud, Centre D'Orsay, France, 1975. RRT is the retention time relative to cholesterol in GLC on 1% of XE-60 on silanized chromosorb W at 235 °C in a 6 ft U-tube.
(13) These conditions have been reported by J. P. Schmit, M. Piraux, and J. F. Pilette, *J. Org. Chem.*, **40**, 1586 (1975), to yield only cholesteryl acetate (81% yield) from the $\Delta^{5,20(22)}$-dienyl acetate prepared by the Wittig reaction with pregnenolone. However, no attempt was made at chromatographic analysis or purification and no spectroscopic data were reported. Not only are the epimers produced in our hands from either the Δ^5- or 3,5-cyclosterol, but we found that reduction of the Δ^5-sterol leads to 28% of the stanol which was apparent both by mass and ^{1}H NMR spectra.
(14) Other examples include 20α- and 20β-hydroxycholesterol, their 17α-hydroxy derivatives, and the (*E*)- and (*Z*)-isomers of 3β-hydroxy-pregn-5,17(20)-diene substituted at C-20 with a CHO or CN group. The upfield shift on passing from the right- to the left-handed isomer is 0.1–0.2 ppm. For further details and a key to the literature see W. R. Nes et al.[11]
(15) The following plants were extracted and the neutral lipid was chromatographed on Al_2O_3 to obtain the sterols which were further separated on a column of lipophilic Sephadex: *Lycopodium complanatum*, the ferns, *Dryopteris noveboracensis* and *Polystichum acrostichoides*, the lower and higher angiosperms, *Ginko biloba* and *Pinus pinea*, the lower angiosperms, *Liriodendron tulipifera* and *Podophyllum peltatum*, and the higher angiosperms, *Pisum sativum*, *Glycine max*, *Brassica oleracea*, and *Kalmia latifolia*. We thank W. D. Nes for the isolation from *K. latifolia* and S. Behzadan for the isolation from *B. oleracea*. All plants yielded 24α-ethylcholesterol (examined separately) and an inseparable mixture of 24α- and 24β-methylcholesterol. In the latter mixture two doublets for C-21 closely spaced (3 Hz) were seen. The ferns also yielded cholesterol as a separate fraction. 24β-Ethylcholesterol derived from the green alga, *Chlorella ellipsoidea* was the gift of G. W. Patterson. Stigmasterol was of commercial origin, presumably from *Glycine max*. Commercial cholesterol examined was presumably from animals. Ergosterol used was commercial and presumably isolated from yeast. In other work, W. R. Nes, J. H. Adler, and M. Young, (Lipids, submitted), we have demonstrated that samples of ergosterol from yeast, *Neurospora crassa*, *Agarigus sp.*, and *Lycopodium complanatum* have identical ^{1}H NMR spectra which is the same as that of commercial ergosterol. Spectral data on some of the sterols mentioned have been published (W. R. Nes, K. Krevitz, and S. Behzadan, *Lipids*, **11**, 118 (1976)) and demonstrated the correctness of the configurational assignment at C-24.
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(17) The apparent inversion of configuration (17β-H-atom in **5** becoming the 20α-H-atom in **3**) is not real and is derived from different conventions of nomenclature in the nucleus and side chain. At C-20 an α-oriented H-atom projects toward the front of the molecule in the right-handed 17(20)-conformer as does the 17β-H-atom.
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The Isolation and Structure of Aplysistatin[1]

Sir:

The toxic effects of sea hare (Mollusca phylum, Aplysiidae family) constituents were well known to various ancient peoples, such as those of the Mediterranean basin.[2] By 150 A.D. such marine animal biosynthetic products had already found application in certain medical treatments.[3] This potentially useful source of medicinal agents seems to have received little attention and has so far nearly eluded modern chemical and biological evaluation. We now wish to report[4] that a 2-propanol extract of the South Pacific Ocean (Australia) sea hare *Aplysia angasi* was found to significantly inhibit (T/C 175 at 400 mg/kg) progression of the National Cancer Institute's murine lymphocytic leukemia P-388 and growth of the new P-388 in vitro cell line. The latter in vitro technique was utilized for guiding isolation procedures.[5]

Detailed chromatographic (prepacked silica gel columns[6]) separation of a chloroform-soluble fraction prepared from the 2-propanol extract gave in a series of fractions eluted by 9:1 ligroin–ethyl acetate a cytotoxic (P-388, ED_{50} 2.7 μg/ml and KB ED_{50} 2.4 μg/ml) component designated aplysistatin (**1**, mp 173–175 °C) with empirical formula $C_{15}H_{21}O_3Br$ (M^+ 330); ORD in methanol $[\alpha]^{25}_{589}$ −375°, $[\alpha]^{25}_{278}$ +21 500, and $[\alpha]^{25}_{270}$ +17 500; CD in methanol [θ]nm + 8580 (259); IR (KBr) 1765, 1676, 1230, 1205, 1010, 1000, 628, and 590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.96 (s, 3 H, methyl), 1.16 (s, 3 H, methyl), 1.28 (s, 3 H, methyl), 1.6–2.4 (m, 5 H, methylene), 2.58 (m, 2 H), 3.9 (m, 2 H), 4.52 (t, J = 8.5 Hz, 1 H), 5.17 (m, 1 H), and 7.00 (m, 1 H).

Single crystals of aplysistatin of suitable size for data collection were obtained from acetone–hexane. On the basis of the observed Laué symmetry and systematic extinctions, the crystal was assigned the orthorhombic space group $P2_1\,2_1\,2_1$: with a = 9.982 (9), b = 7.182 (2), c = 20.586 (9) Å; Z = 4; ρ_{calcd} = 1.482 g/cm^3 for $C_{12}H_{21}O_3Br$, ρ_{obsd} = 1.469 g/cm^3. Diffraction intensities were measured in the θ–2θ scan mode using graphite monochromated Mo Kα radiation on a Syntex $P\bar{1}$ autodiffractometer; of the 2107 reflections examined ($2\theta \leq 55°$) a total of 1967 unique reflections were retained with $|F_o| > 0$. Corrections were made for the absorption of Mo Kα radiation,[7] and there was no observable extinction in the crystal.

The structure was solved by standard heavy atom methods.[8] A comparison was made of large block least-squares refinements (172 independent variables in two blocks) of the two structural configurations with anisotropic thermal parameters and fixed hydrogen positions using the anomalous scattering factors for Br, O, and C.[9] The standard residuals at convergence were R = 0.1018 and R = 0.0945, respectively, for the two models and the weighted residuals $R_w = (\sum_w(|F_o| - |F_c|)^2/\sum_w|F_o|^2)^{1/2}$ of 0.0719 and 0.0649, respectively, were obtained for $w = 1/\sigma_F^2$.

The perspective view shown in Figure 1 displays all the essential conformational and configurational features of the

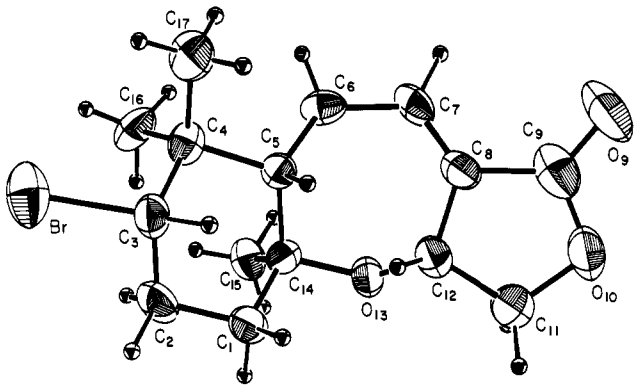
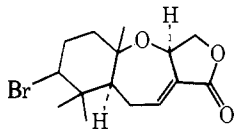


Figure 1.

aplysistatin[10] molecule. The four chiral centers are C_3 (*S*), C_5 (*S*), C_{12} (*R*), and C_{14} (*S*).



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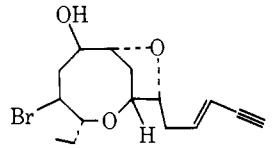

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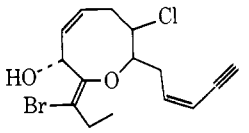

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Since sea hares generally depend upon marine algae for nutrition it may be useful to consider such an exogenous primary source of aplysistatin. In this respect the isomeric substance laurefucin (**2**) has been isolated from the Japanese marine algae *Laurencia nipponica* Yamada[11] and the related chondriol (**3**) has been obtained from the marine algae *Chondria oppositiclada* Dawson.[12] Presently the antineoplastic effects of aplysistatin are being assessed in the National Cancer Institute's laboratories and we are investigating other new substances from sea hares.

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References and Notes

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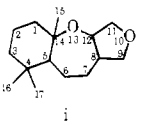
(10) Aplysistatin represents a new type of sesquiterpene and oxygen heterocyclic system. For this new ring system we suggest the name aplysistane and the numbering system presented by structure i.



i

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Selenium Stabilized Anions. Synthetic Transformations Based on Propargyl Selenoxides

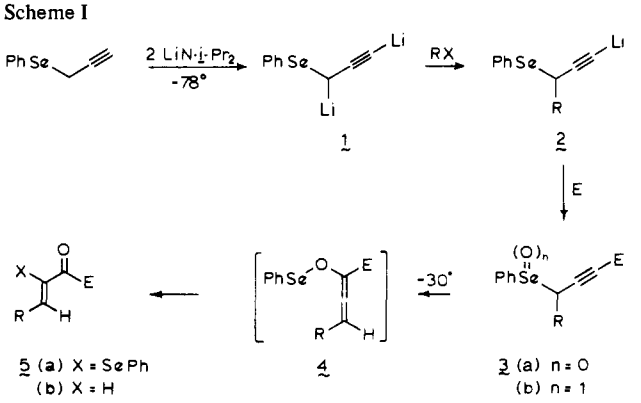
Sir:

Heterosubstituted propargyl and allenyl organolithium reagents have potential as synthetic precursors to highly functionalized 3-carbon fragments.[1] We report here the preparation of the dianion **1**,[2] and reactions of the propargyl selenides derived from it (Scheme I).

Phenyl propargyl selenide is rapidly deprotonated by 2 equiv of lithium diisopropylamide in tetrahydrofuran or glyme at −78 °C to give a pale yellow solution of the dilithium reagent **1**.[3] Alkyl halides react with **1** exclusively at the α position (>99.5% α-methylation), primary bromides and iodides at −78 °C, isopropyl iodide at −40 °C (Table I). The resulting acetylenic lithium reagent **2** can then be protonated, alkylated, or treated with a variety of other electrophiles (E) to give 1,3-disubstituted propargyl selenides, **3a**, usually in excellent yield.

A number of useful transformations of **3a** can be envisaged. Oxidation gives the selenoxide **3b**, which rearranges to α-phenylselenoenone (**5a**)[4] at −40 to −30 °C, presumably via **4**.[5] The table shows that a variety of complex enones can be quickly assembled using this technique, including otherwise

Scheme I



5 (a) X = SePh (b) X = H **4** **3** (a) n = 0 (b) n = 1