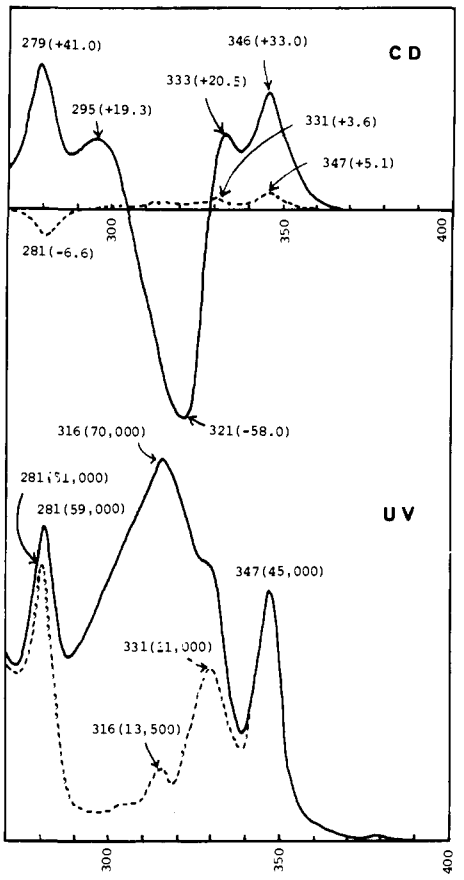


**Figure 2.** Measurements in $CHCl_3$. UV and CD curves of tetrahydrodiol **6** (- - -) and its dma-dibenzoate **7** (—). Only regions above 270 nm are shown due to difficulty in measurements. The absorption centered at 281 and 331 nm are due to the longitudinal and transverse transitions of the pyrene moiety, respectively.

indicated a diastereoisomeric relationship.[1,3] The later eluting product (designated peak 3 in ref 1) and the in vivo product were identical. We have now separately converted **2a** and **2b** into the guanosine adducts **4** via oxidation to **3**, reaction with poly(G), and hydrolysis, and found that the product derived from **2a** corresponded to the in vivo peak **3** material. Hence the absolute configuration of the in vivo product is represented by **4**.

A second in vivo product was found which corresponded to the minor component (designated peak 2 in ref 1) resulting from hydrolysis of the racemic **3**-modified poly(G).[1] Recent HPLC studies[18] indicate that this in vivo product is derived from the same enantiomer of **3** and that it is probably the corresponding 9,10-cis addition product.[4,19]

The formation of adducts from only one enantiomer of **3** in vivo[1] is consistent with recent evidence that only **3** derived from **2a** is formed during the in vitro microsomal oxidation of **1**.[20] Nucleic acids themselves are highly asymmetric and hence, the absolute stereochemistry of these in vivo adducts is intimately related to their interaction with and modification of nucleic acid structure.[21]

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(12) The menthoxy derivatives with $[\alpha]_D$ values of −300° and +160° gave, after hydrolysis and recrystallizations from $CCl_4$, **2a** $[\alpha]_D$ −460° and **2b** $[\alpha]_D$ +460°, respectively.

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## The Stereochemistry of Sterols at C-20 and Its Biosynthetic Implications

*Sir:*

While the three-dimensional character of the dominant sterols[1] in biological systems has been well established in terms of absolute configurations at the various asymmetric centers in the nucleus[2] and more recently at C-24,[3-9] conformational isomerism of the side chain in all sterols[10] and the configuration at C-20 in most of them have remained enigmatic. Rotation about the 17(20)-bond is especially interesting, because in the

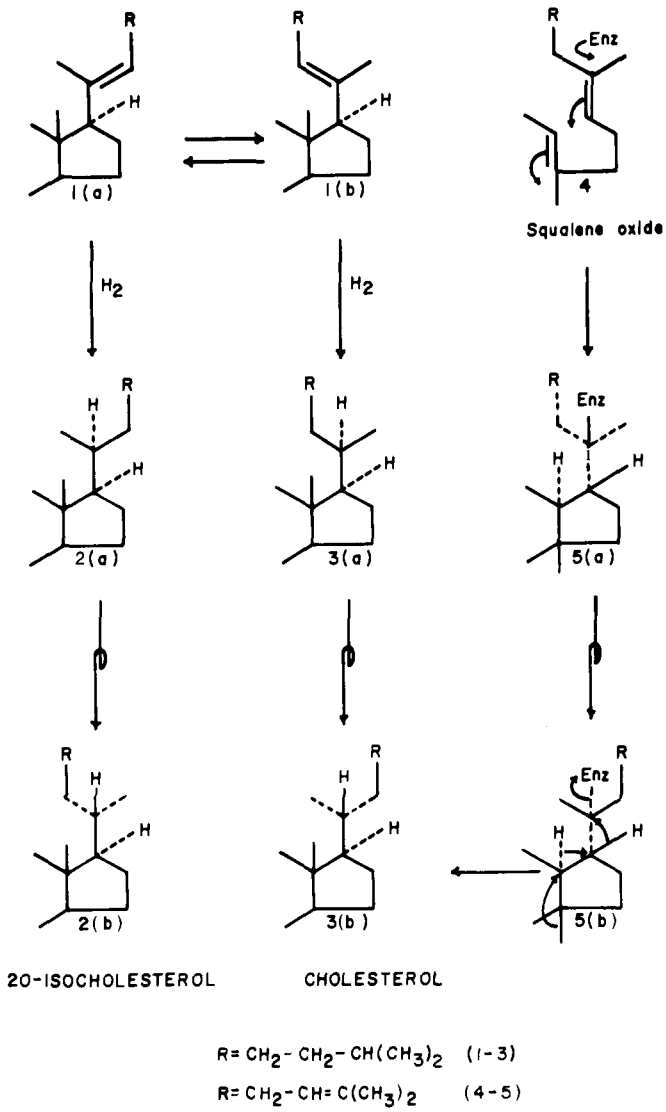
different isomers the bulky six carbon atoms comprising C-22 through C-27 (and C-28 and C-29 as the case may be) are placed in very different locations relative to the tetracyclic nucleus.

Examination of molecular models reveals that the side chain should be the most stable with C-22 either to the left (cis oriented with respect to C-13) (**3a**) or to the right (**3b**) in the conventional view of the molecule. A choice in favor of the right-handed conformation can be made in the following way. In animal cholesterol for which the configuration at C-20 is known[2] the H atom at C-20 will project pseudo-axially toward and C-21 and C-22 pseudo-equatorially away from the observer when C-22 is to the right in a skew conformation (**3b**) about the 17(20)-bond. In the left-handed conformation (eclipsed, **3a**), C-21 and C-22 will lie pseudo-equatorially toward the observer and therefore closer to ring D. Steric compression between C-21 and C-22 and the ring should destabilize this conformer relative to the right-handed one. This has recently been demonstrated by the hydroboration of (*Z*)-17(20)-dehydrocholesterol.[11] The left-handed conformer of 20$\alpha$-hydroxycholesterol must have arisen, but it was the right-handed conformer found as the product. We have now confirmed the theoretical analysis in a different way, viz., by spectral studies of cholesterol and its epimer, 20-isocholesterol. In the epimer (with reverse three-dimensional characteristics at C-20) it should be the left-handed conformer (**2b**) which is skew and which possesses the least steric compression, i.e., an opposite conformational preference compared to cholesterol, 20$\alpha$-hydroxycholesterol, and other sterols of the same configuration. This was observed experimentally.

(*E*)-20(22)-Dehydrocholesterol[12] (**1**), used as the 3,5-cyclocholest-6$\beta$-yl methyl ether to protect the $\Delta^5$-bond, was reduced with hydrogen and platinum oxide in dioxane containing a small amount of acetic acid.[13] The product at this and subsequent stages of isolation on examination by glc was a 1:1 mixture of the C-20 epimers.[13] Retro-3,5-cyclosteroid rearrangement (zinc acetate and acetic acid), hydrolysis of the acetates, and separation of the resulting epimeric sterols on a column of $Al_2O_3$ deactivated with 10% of water gave cholesterol (**3b**): mp 146–147 °C; $^1$H NMR $\delta$ 0.69 (s, C-18 protons), 1.02 (s, C-19 protons), 0.88 (d, $J$ = 6–7 Hz, C-26 and C-27 protons), and 0.91 (d, $J$ = 6–7 Hz, C-21 protons); and 20-isocholesterol (**2b**): mp 153–154 °C; RRT 0.91; $^1$H NMR $\delta$ 0.69 (s, C-18 protons), 1.03 (s, C-19 protons), 0.88 (d, $J$ = 6–7 Hz, C-26 and C-27 protons), and 0.81 (d, $J$ = 6–7 Hz, C-21 protons).

The existence of opposite conformational preferences in the two tetrahedral products (**3b** and **2b**) is demonstrated by the upfield shift (0.10 ppm) in the signal for C-21 for 20-isocholesterol compared to cholesterol. Such a shift is predicted from an analysis of the spectra of, among others, (*E*)- and (*Z*)-17(20)-dehydrocholesterol in which C-22 is fixed rigidly to the right and left, respectively, by virtue of the 17(20)-double bond.[14] The formation of both cholesterol and 20-isocholesterol by reduction of the $\Delta^{20(22)}$-sterol also demonstrates the presence of the two rotational isomers (**1a** and **1b**) in the starting material. Since the ratio of the products was 1:1 the positioning of the R group (in **1**) to the right or left can have had no appreciable effect on the energies of the respective transition states. This implies by analogy that the placing of R on the right or left in the final sterol (and, ignoring the double bond, also in the initial $\Delta^{20(22)}$-sterol) has no appreciable effect in terms of the bulk of the R group. The ratio of the reduction products therefore further confirms that the conformational preferences in the epimeric sterols must be determined by the configuration at C-20.

Since inversion of the configuration at C-20 necessarily results in conformational inversion which is accompanied by a predictable change[14] in the signal for C-21, $^1$H NMR spectroscopy becomes a method for determination of the configuration at C-20. Consequently, we have examined a large number of samples of cholesterol and its 24-methyl- and 24-ethyl derivatives as well as derivatives of ergosterol and stigmasterol derived from algae, fungi, tracheophytes, and animals.[15] In every case for the $\Delta^5$-sterols the signal from C-21 was at 0.91–0.93 ppm. This is the same as found for cholesterol, and it is 0.10 downfield from the position found in 20-isocholesterol. Similarly, 24$\beta$-methylcholesterol derived synthetically from ergosterol exhibits a doublet at 0.92 ppm. The configuration at C-20 must therefore be the same (20$\alpha$-H) in all cases.




The results are consistent with the involvement of a previously suggested[16] intermediate (**5a**) in the cyclization of squalene oxide (**4**) in which a group probably from the cyclase has attacked the front of C-20 (the opposite side of the $\Delta^{17(20)}$-double bond from the side attacked by C-13) thus permitting time for rotation (**5a** to **5b**) to occur about the 17(20)-bond prior to migration of the 17$\beta$-H-atom to C-20 (**5b** to **3b**). Examination of molecular models reveals an explanation for this phenomenon. At the stage in which the protosterol is complexed with the enzyme (or other attacking species) the least stable rotamer about the 17(20)-bond, and the one necessarily resulting from cyclization due to the trans-oriented double bond in squalene oxide (**4**), is the one in which C-22 lies to the left with the 17$\beta$-H-atom and the substituent (enzyme, etc.) opposed (pseudo-1,2-diaxial) in an eclipsed conformation (**5a**). A large substituent such as the enzyme might also ex-

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.

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(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 $[\theta]$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; $\rho_{calcd}$ = 1.482 g/cm$^3$ for $C_{12}H_{21}O_3Br$, $\rho_{obsd}$ = 1.469 g/cm$^3$. Diffraction intensities were measured in the $\theta$–$2\theta$ 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