predicts only a DPA or stilbene excited state. The rubrene emission could possibly arise from some type of resonant transfer (such as Förster's transfer)[20] from the DPA or stilbene excited state. However, we have been unable to demonstrate Förster's transfer photochemically for these mixed systems. Also, Cruser and Bard found no evidence for Förster's transfer in mixed rubrene–DPA annihilation studies.[21] Furthermore, similar results are obtained for mixtures of fluoranthene and $DPACl_2$, where there is virtually no overlap between the fluoranthene absorption and the DPA emission spectrum. The fact that virtually all the luminescence osbserved for aromatic mixtures is that of the more easily reduced species cannot easily be explained on the basis of collisional electron or excited-state energy-transfer processes either.

As the direct (two-electron) electroreduction of $DPACl_2$ to DPA at -1.1 V does not yield ecl, it appears that the homogeneous reduction of $DPACl_2$ by electrogenerated $R\cdot^-$ goes by a distinctly different reaction path (yielding R*) than the electrochemical reduction. The reduction by $R\cdot^-$ probably involves two one-electron steps with intermediate chemical step(s).

As the high efficiency of this chemiluminescence reaction is of fundamental interest and appears to be general (a wide variety of aryl and alkyl halides[16b] exhibit similar behavior), and the "apparent" energy transfer phenomenon is totally unexpected, further study of this reaction is now in progress. This cl and the energy-transfer processes will be easier to study quantitatively as both transient and steady-state luminescence can be attained.

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Cis Reduction of Δ^{24} of Lanosterol in the Biosynthesis of Cholesterol by Rat Liver Enzymes. A Revision

Sir:

For the definition of the overall (cis or trans) mechanism of reduction of Δ^{24} of lanosterol (**1**) in the biosynthesis of cholesterol (**2**) the prochirality at C-24 and C-25 of the produced cholesterol has to be determined. We have proven that in cholesterol biosynthesized in the S-10 fraction of rat livers a 24-pro-*S* proton is added.[1,2] The methyls at C-25 are derived from C-2 and from C-3′ of mevalonic acid (MVA).[3] To differentiate the two methyls we incubated cholesterol, biosynthesized from [2-^{14}C]MVA, with *M. smegmatis*,[4,5] isolated the 26-hydroxycholest-4-en-3-one[6] (**3**), and degraded it to 26-norcholestenone (**4**). The conversion of **3** to **4** involved the loss of *ca.* 0.8 atom of ^{14}C and revealed that the oxygen function resided on the methyl derived from C-2 of MVA.[6] To define the absolute configuration at C-25, comparison samples of 26-hydroxycholest-4-en-3-one (**3**) were prepared from kryptogenin and by various stereoselective procedures,[6] and we compared their specific rotations $[\alpha]_D$ with that of the microbial specimen.

We noticed that the $[\alpha]_D$ (measured on a Hilger MKIII instrument) of the microbially prepared sample of **3** was similar to that derived from kryptogenin and was more positive than that of the 25*RS* specimen (Table I, column A). The synthetic 25*R* and 25*S* specimens of **3** were more and less positive, respectively, than the 25*RS* samples (Table I, column A). Since kryptogenin has the 25*R* configuration,[7] we concluded that the microbial sample also has the 25*R* configuration. Because the geometry at Δ^{24} of lanosterol[8] is as in **1**, we inferred that the reduction of Δ^{24} entails a trans addition of two hydrogens.[6] We noted that our conclusions are at variance with the situation in tigogenin[6] and were in contrast with recent observations of Professors Ch. Tamm and D. Arigoni on the biosynthesis of sapogenins in *D. lanata*.[9] To clarify the discrepancy we first repeated the measurements of the specific rotations of the various samples of **3**, this time using a Rudolph and Sons photoelectric polarimeter (Table I, column B). Although the absolute $[\alpha]_D$ values differ somewhat from the results obtained with the Hilger MKIII polarimeter, clearly the relative situation remained unchanged. To solve conclusively the problem of the absolute configuration we carried out an X-ray diffraction analysis of the 26-*p*-bromobenzoate ester (mp 107–109°, *m/e* 582, 584; M^+) derived from the microbially prepared **3**.

The para bromobenzoate derivative crystallizes as flat plates in the monoclinic system, space group $P2_1$

Table I. Specific Rotation $[\alpha]_D$ (Degrees) of 26-Hydroxycholest-4-en-3-ones

Origin and method of preparation	$[\alpha]_D$ in chloroform A[a]	B[b]
Asymmetric hydroboration[c] with (+)-diisopinocampheylborane	+87.4; +86.0	+89.6
From kryptogenin	+84.45; +85.6	+92.7
Incubation of chesterol with *M. smegmatis*	+87.1; +86.1	+89.4
Symmetric hydroboration[c] with disiamylborane	+80.35	+85.6
Asymmetric hydroboration[c] with (−)-diisopinocampheylborane	+74.8	+78.8

[a] Measured using a Hilger MKIII polarimeter. [b] Measured using an O. C. Rudolph and Sons, Inc., photoelectric polarimeter with oscillating polarizer. [c] Hydroboration of cholest-5,26-dien-3β-ol.

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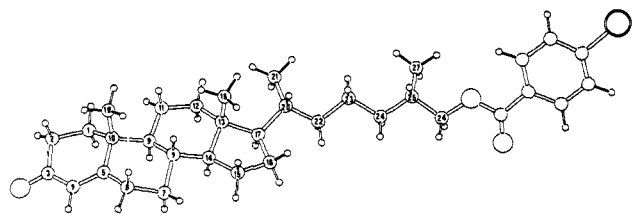
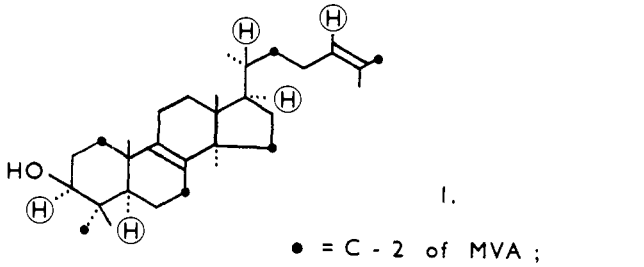


Figure 1. Drawing of 26-hydroxycholestenone *p*-bromobenzoate from X-ray results. The molecule is shown in projection down the *b* axis of the crystal.

with $a = 10.80$, $b = 9.86$, and $c = 14.52$ Å, and $\beta = 91.0°$. Three-dimensional X-ray diffraction intensity data (3274 reflections) were gathered on a computer-controlled diffractometer using nickel-filtered Cu Kα radiation. The data were corrected for systematic errors including absorption.[10] A trial structure was determined by the heavy atom method. The asymmetric unit of a three-dimensional electron density map, phased using the Br position found by Patterson analysis, contained images of two superimposed molecules, as expected. In this analysis, however, separation of the images was quite straightforward. Atomic positions and first isotropic, then anisotropic, thermal parameters were refined by least-squares techniques. Hydrogen positions were checked by difference Fourier techniques. The agreement factor R $(=\Sigma||F_o| - |F_c||/\Sigma F_o)$ is 0.069.

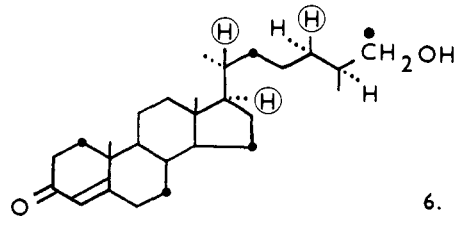
A drawing of the molecule prepared directly by computer from current crystallographic positions[11] is shown in Figure 1. The configuration is clearly 25*S* and not as previously inferred[6] 25*R*. The reason for the abnormality of the specific rotations (Table I) is under investigation.



I.

● = C - 2 of MVA ;

Ⓗ = 4 - pro - R. 4 of MVA.



6.

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(11) A listing of atomic coordinates will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Reprint Department, ACS Publications, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to author, title of article, volume, and page number. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche.

We have proven that in the 25*S*-26-hydroxycholest-4-en-3-one obtained by microbial oxidation of cholesterol the carbon derived from C-2 of MVA bears the oxygen function.[6] Consequently the absolute configuration at C-24 and -25 is as in **6**. Since the geometry at Δ^{24} of lanosterol is as in **1**, it follows that the reduction of this olefinic bond in the biosynthesis of cholesterol in the S-10 fraction of rat livers entails a cis addition of hydrogens at C-24 and -25 as indicated in **6** and is *not* a trans reduction.[6]

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Carbon-13 Fourier Transform Nuclear Magnetic Resonance Spectroscopy. II.[1] The Pattern of Biosynthetic Incorporation of [1-^{13}C]- and [2-^{13}C]Acetate into Prodigiosin[2]

Sir:

We wish to report the first carbon-13 Fourier transform (FT) nmr study of the detailed labeling pattern in a metabolite biosynthetically enriched with ^{13}C. Spectra were determined using a computer controlled FT system for ^{13}C.[1]

No detailed pattern of incorporation of biosynthetic substrates into prodigiosin (I) has yet been reported. In part, this reflects the difficulties involved in isolating substantial yields of fragments in the chemical degradation of the prodigiosin molecule. Use of ^{13}C nmr represents a potent method which helps circumvent the need for chemical degradation. Recent studies have employed both ^{13}C–^{1}H satellite nmr spectroscopy[3] and ^{13}C cw-nmr[4] to assign biosynthetic labeling patterns. Fourier transform nmr, as used in this study, provides substantially greater sensitivity, which has allowed us to use ^{13}C single resonance spectra as an aid to assignment, and to work with small samples.

Prodigiosin (I), isolated from the bacterium *Serratia marcescens*, is one of a series of bacterial secondary metabolites having in common an unusual system

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