$10^{10}$ $s^{-1}$ line obviously overestimates the $T_1$ values of 1,2-decanediol while the $D_i = 1 \times 10^9$ $s^{-1}$ line gives a much closer fit, *although no frequency dependent $T_1$ values are predicted.* However, the presence of a relatively narrow distribution ($p = 50$)[15] with $D_i = 1 \times 10^{10}$ $s^{-1}$ provides a much better fit at 67.9 MHz and also *predicts the requisite field dependence,* albeit only qualitatively.

The value of the correlation time required to describe the overall molecular motion is consistent with the fact that the molecule is extensively hydrogen bonded, the correlation time being about an order of magnitude greater than that observed for decane.[14a] Significantly, the diffusion coefficients along the chain in 1,2-decanediol are in close agreement with the values obtained by Levine et al.[14a] for decane.

The qualitative agreement between calculated and observed $T_1$s at the two fields using both the distribution and multiple internal rotation cannot be improved upon without introducing additional factors. The observed field dependence is too great for the longer relaxation times to be quantitatively predicted. Apparently, the assumption of independent rotation by a diffusional process about the carbon–carbon bonds is inadequate. The calculated field dependence could be increased by taking into account the interdependence of the conformational changes possible along the chain.[14b] (The motions would necessarily be more correlated.) It is apparent that the published theory[13,14] which finds that five successive methylene carbons are sufficient to decouple effectively chain segments is *not* valid for decanediol.

1-Decanol is also extensively hydrogen bonded in neat solution.[7] The $^{13}C$ $T_1$s of 1-decanol given also in Table I do not show a significant field dependence. The two field $T_1$ data for decanol at 36 °C thus do not require invocation of a complex cooperative motional description as in 1,2-decanediol.

The nuclear Overhauser enhancement factors (NOEF) were ~1.90 for all carbons of 1,2-decanediol, the theoretical limit being 1.988. The predicted NOEFs for the carbons using the distribution modified by the multiple internal rotations are also in this region. This parameter is not a sensitive indication of the motional model under these circumstances. Decanol also exhibits maximum nuclear Overhauser enhancements but no $T_1$ field dependence.

**Conclusions.** All of the $T_1$ field dependences for 1,2-decanediol can be explained with a model of at least partially cooperative segmental motions superimposed on an overall molecular reorientation having some slow ($\gtrsim 10^{-9}$ s) components. The slower motions are incompletely decoupled from the segmental motions of the chain carbons, including the C-10 $CH_3$ group.

*The implications of this preliminary study may be far reaching.* One of the strengths of $^{13}C$ $T_1$ measurements is an ability to probe molecular motion at individual carbon sites. A weakness has been the inability to generally probe cooperativity or couplings of group internal motions. The variable field approach can probe motions as in the segmental chain motion of 1,2-decanediol. We are now investigating further this situation and its application to the study of segmental motions in model membrane structures.

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**George C. Levy,*[16] Mary Pat Cordes**
**John S. Lewis, David E. Axelson**
*Department of Chemistry*
*The Florida State University*
*Tallahassee, Florida 32306*
*Received February 18, 1977*

## 14α-Ethyl-5α-cholest-7-ene-3β,15α-diol, an Extraordinarily Potent Inhibitor of Sterol Biosynthesis in Animal Cells[1]

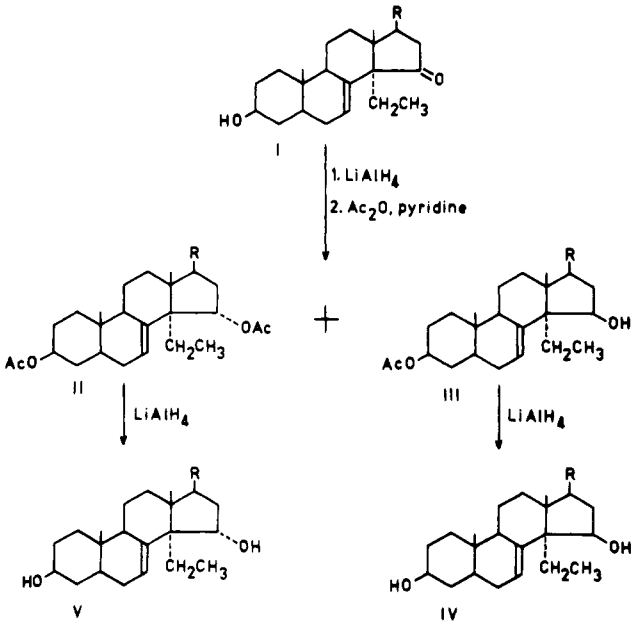
*Sir:*

A number of oxygenated derivatives of cholesterol have been shown to act as potent inhibitors of sterol biosynthesis in animal cells in culture.[2-4] The site of the inhibitory action of these compounds appears to be at the level of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a key enzyme in the regulation of sterol biosynthesis which is responsible for the formation of mevalonic acid.[2-4] The most potent inhibitor of this type reported to date is 25-hydroxycholesterol which caused a 50% reduction of sterol synthesis in L cells and in primary cultures of liver cells at concentrations of $7.0 \times 10^{-8}$ M and $1.0 \times 10^{-6}$ M, respectively. Recently we have found that a number of 15-oxygenated sterols also act as inhibitors of sterol synthesis in the same cell culture systems.[5] We have also found that subcutaneous administration to rats of one of these compounds, 5α-cholest-8(14)-en-3β-ol-15-one, results in a reduction in serum cholesterol levels and an inhibition of hepatic sterol synthesis.[6]

The purpose of this communication is to describe the chemical syntheses of 14α-ethyl-5α-cholest-7-ene-3β,15β-diol and 14α-ethyl-5α-cholest-7-ene-3β,15α-diol and to report that the latter compound is an extraordinarily potent inhibitor of sterol biosynthesis in both L cells and in primary cultures of fetal mouse liver cells.

5α-Cholest-8(14)-en-3β-ol-15-one, prepared as described previously,[6] was alkylated with ethyl iodide in the presence of potassium *tert*-butoxide in *tert*-butyl alcohol to give, after purification by medium pressure silica gel column chromatography and crystallization from acetone–water, 14α-ethyl-5α-cholest-7-en-3β-ol-15-one (I)[7] in 42% yield. Reduction of I with lithium aluminum hydride in ether gave a 70:30 mixture of 14α-ethyl-5α-cholest-7-ene-3β,15α-diol and 14α-ethyl-5α-cholest-7-ene-3β,15β-diol (89% yield) which could not be resolved by TLC on silica gel G or on GLC on OV-1 or OV-17 columns but which could be separated, in the form of the bis-

TMS derivative, by GLC on OV-1 or OV-17 columns. The epimeric mixture, upon treatment with acetic anhydride in pyridine at room temperature for 24 h, gave, after medium-pressure silica gel column chromatography and crystallization from acetone–water, bis(3β,15α-acetoxy)-14α-ethyl-5α-cholest-7-ene (II,[8] 55% yield) and 3β-acetoxy-14α-ethyl-




5α-cholest-7-en-15β-ol (III,[9] 21% yield). Reduction of III with lithium albumin hydride in ether gave, after crystallization from acetone–water, 14α-ethyl-5α-cholest-7-ene-3β,15β-diol (IV[10]) in 93% yield. Reduction of II with lithium aluminum hydride in ether gave, after crystallization from acetone–water, 14α-ethyl-5α-cholest-7-ene-3β,15α-diol (V[11]) in 91% yield.

While x-ray crystallographic analyses of the compounds in question have not been made, the assignments of configuration at C-14 and C-15 can be made with a high degree of confidence. Evidence for the trans configuration of C–D ring juncture in the various 14-ethyl-substituted compounds is derived primarily by analogy to the corresponding 14-methyl compounds prepared in the same fashion in which the configuration at C-14 has been unequivocally established by x-ray crystallography.[12] In addition, in I, from which the other 14-ethyl compounds were prepared, the NMR spectral results provide strong evidence in support of a trans-C–D ring juncture. The resonance due to the C-7-proton in I occurs at 6.52 ppm in close agreement with the corresponding resonance in 3β-benzoyloxy-14α-methyl-5α-cholest-7-en-15-one (6.55 ppm)[12b] and in 14α-methyl-5α-cholest-7-en-3β-ol-15-one (6.61 ppm).[5b] In the latter cases the absolute configuration of the 14α-methyl function is known from the results of x-ray analysis.[12] In these $\Delta^7$-15-ketosterols with the trans-C–D ring juncture there is a substantial deshielding of the C-7 proton by the 15-ketone function. This deshielding has been noted to be absent in the case of a $\Delta^7$-15-ketosterol with the cis-C–D ring juncture. The resonance due to the C-7 proton in 3β-benzoyloxy-5α,14β-cholest-7-en-15-one occurs at 5.48 ppm.[13] The absolute stereochemistry of the C–D ring juncture of the latter compound has been established by x-ray crystal analysis of a suitable derivative.[13a,14] The absolute configurations of the 15-hydroxy functions in IV and V are indicated by the results of NMR and optical rotation studies. The observed vicinal coupling constants for the C-15 H in IV and V are in close agreement with the calculated[15] values for the 15α ($J$ = 0, 6.2 Hz) and 15β hydrogens, ($J$ = 6.2, 8.5 Hz), respectively. Additional evidence in support of the assigned configurations was derived from considerations of the optical rotations of IV and V. In steroids with the trans-C–D ring juncture, it has been reported that the 15β-hydroxy derivatives are more levorotatory than the corresponding 15α-hydroxy derivatives.[16-18] In the case under consideration the compound in which the 15-hydroxy function was assigned the 15α configuration had a specific rotation of +9.8° while that assigned the 15β configuration had a specific rotation of −41.8°.

**Table I**

| Inhibitor | Concentrations (μM) required for 50% inhibition of sterol synthesis[a] in: L cell cultures | Primary cultures of mouse liver cells |
|---|---|---|
| 14α-Ethyl-5α-cholest-7-ene-3β,15β-diol (IV) | 0.4 | 0.8 |
| 14α-Ethyl-5α-cholest-7-ene-3β,15α-diol (V) | 0.05 | 0.06 |
| 25-Hydroxycholesterol (ref 2) | 0.07 | 1.0 |

[a] Primary cultures of fetal mouse liver cells and mouse L cell cultures were grown in serum-free media as described previously.[1,2] The preparation of steroid-containing media and the procedures for assay of the rate of conversion of [1-$^{14}$C]acetate into digitonin-precipitable sterols and fatty acids were as described previously.[1,2] L cell cultures were preincubated with steroids for 4 h; then [1-$^{14}$C]acetate was added at a concentration of 4 μmol (4 μCi)/mL. Conditions for incubating steroids with liver cell cultures were similar to those described for L cell cultures except that the cultures were incubated with the test steroids for 12 h before labeled acetate was added. Steroids were tested over a range of at least four concentrations and the assay was repeated until the concentration of steroid required to produce 50% inhibition was located on the steeply declining part of an activity vs. concentration plot. To diminish the effects of generalized differences in cellular metabolism, rates of sterol synthesis from [1-$^{14}$C]acetate were calculated as the ratio of [$^{14}$C]sterols to [$^{14}$C]fatty acids.[1,2]

Both IV and V were found to be potent inhibitors of sterol biosynthesis in both L cell (mouse fibroblast) cultures and in primary cultures of mouse fetal liver cells (Table I). The 15α-hydroxy epimer was considerably more active than the 15β-hydroxy compound. While V had activity comparable with that of 25-hydroxycholesterol in L cells, it was considerably more potent than the 25-hydroxysterol in the inhibition of sterol biosynthesis in the liver cells. None of the sterols shown in Table I inhibited the rate of fatty acid synthesis or the rate of $CO_2$ production in either cell type. Since liver constitutes a major site for the biosynthesis of cholesterol in higher animals, the high potency (50% inhibition of sterol synthesis at $6 \times 10^{-8}$ M) of V in the liver cell culture system is noteworthy.

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(8) II: mp 171.5–172.5 °C; single component on TLC and GLC; mass spectrum (rel intensity) 514 (M, 4%), calcd for $C_{33}H_{54}O_4$ 514.4022, found 514.4033; NMR 2.03 (s, 6 H, methyls of acetate functions), 4.72 (m, 1 H, C-3 H), 5.15 ppm (m, 2 H, C-7 H and C-15 H); $[\alpha]_D$ +4.4° ($CHCl_3$).
(9) III: mp 135–136 °C; single component on TLC and GLC; mass spectrum (rel intensity) 472 (M, 10%), calcd for $C_{31}H_{52}O_3$ 472.3916, found 472.3888; NMR 2.03 (s, 3 H, methyl of acetate function), 4.10 (m, 1 H, C-15 H), 4.74

(m, 1 H, C-3 H), 5.63 ppm (m, 1 H, C-7 H); $[\alpha]_D$ −44.1° ($CHCl_3$).
(10) IV: mp 83.5–84.5 °C; single component on TLC and GLC (and on GLC of the bis-TMS derivative); mass spectrum (rel intensity) 430 (M, 9%), calcd for $C_{29}H_{50}O_2$ 430.3811, found 430.3820; NMR 3.55 (m, 1 H, C-3 H), 4.10 (d, 1 H, $J$ = 6.5 Hz, C-15$\alpha$ H), 5.60 ppm (m, 1 H, C-7 H); $[\alpha]_D$ −41.8° ($CHCl_3$).
(11) V: mp 202–203 °C; single component on TLC and GLC (and on GLC of the bis-TMS derivative); mass spectrum (rel intensity) 430 (M, 3%), calcd for $C_{29}H_{50}O_2$ 430.3811, found 430.3811; NMR 3.62 (m, 1 H, C-3 H), 4.34 (doublet of doublets, 1 H, $J$ = 6.5, 8.0 Hz, C-15$\beta$ H), 5.52 ppm (m, 1 H, C-7 H); $[\alpha]_D$ +9.8° ($CHCl_3$).
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(19) To whom inquiries should be directed at Rice University.

**G. J. Schroepfer, Jr.,*[19] E. J. Parish**
*Departments of Biochemistry and Chemistry*
*Rice University, Houston, Texas 77001*

**A. A. Kandutsch**
*The Jackson Laboratory, Bar Harbor, Maine 04609*
*Received April 18, 1977*

## Streptonigrin Biosynthesis. 1. Origin of the 4-Phenylpicolinic Acid Moiety

*Sir:*

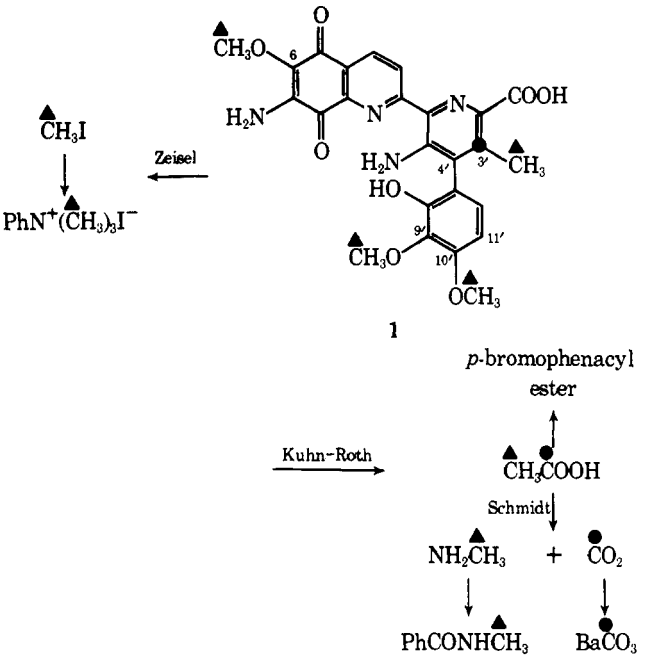
Streptonigrin (**1**), an antibiotic produced by *Streptomyces flocculus* ATCC 13257,[1,2] has striking anticancer properties[3] and is the most potent bone marrow depressant drug known.[4] The structure was originally deduced from chemical studies[5] and was recently confirmed by x-ray crystallography[6] and by $^{13}C$ NMR.[7] Studies on its mode of action,[8] as well as on numerous efforts toward total synthesis[9] and toward synthesis of analogues[10] of the quinoline quinone portion, have appeared.

Russian workers[11] have previously demonstrated that the four methyl groups of **1** are derived from methionine, but the biogenesis of the ring system has not been elucidated.

We now report that tryptophan is the precursor of the unusual 4-phenylpicolinic acid C–D ring system of streptonigrin. Data is also presented on the timing of the four methylation reactions.

Feedings were conducted in 1-L Erlenmeyer flasks containing 250 mL of a complex nutrient broth.[12] These were inoculated with *S. flocculus* spores and shaken at 28 °C. On the third day labeled precursors were added and the broths were worked up[12] 2 days later. After spectrophotometric determination of the amount of streptonigrin produced (UV max 380 nm), authentic streptonigrin (50–100 mg) was added and recrystallized to constant specific activity.

Scheme I[a]




[a] Labeling pattern of streptonigrin: ●, [3-$^{14}$C]tryptophan; ▲, [$^{14}CH_3$]methionine and [3-$^{14}$C]serine.

Degradations of streptonigrin used to locate the labeled positions are shown in Scheme I. Table I lists the precursors fed and the percentage of radioactivity from the streptonigrin found in each degradation product.

[3-$^{14}$C]Tryptophan was very well incorporated into streptonigrin. Surprisingly, all of the activity was located at C-3′ of the pyridine ring. In confirmation of the Russian work,[11] we have found that methionine labels the pyridine methyl group. Additionally, [3-$^{14}$C]serine, a major donor to the one-carbon metabolic pool,[13] labels the methoxyls much more heavily than the *C*-methyl group. This would indicate that C-methylation is occurring at an earlier stage than O-methylation.

Since the previously reported work[11] only identified 87% of the activity from the methionine incorporation in the methyl groups, we have fed [$^{13}CH_3$]-L-methionine (0.28 mmol, 90% enriched) into five 2-L flasks, each containing 500 mL of fermentation broth. The combined broths were extracted and worked up to yield 35 mg of streptonigrin. The $^{13}C$ NMR spectrum indicated significant enrichment of only the four methyl carbons ($I_e/I_u$):[14] C-3′ $CH_3$ (2.1), C-6 $OCH_3$ (0.9), and the C-9′ and C-10′ $OCH_3$s (1.1 each). No other single carbon is significantly labeled by methionine.

Although only 60–70% of the radioactivity of **1** obtained

**Table I.** Incorporation of Labeled Precursors into Streptonigrin[a,b]

| Precursor added[c] | % Incorpn | % of streptonigrin activity in: Phenacyl ester | $BaCO_3$ | $PhCONHCH_3$ | $PhN^+Me_3I^-$ [d] |
|---|---|---|---|---|---|
| [$^{14}CH_3$]-L-Met | 3.7, 3.2 | 21.3 | 4.1 | 20.4 | |
| [3-$^{14}$C]-DL-Try | 7.5, 5.5 | 100 | 60–70 | 0 | |
| [3-$^{14}$C]-DL-Ser | 1.2 | 3.4 | | | 96 |
| [3-$^{14}$C]-DL-Ser | 0.3 | | 2.0 | 5.1 | 81 |
| [3-$^{14}$C]-DL-Phe | 0.005 | | | | |
| [3-$^{14}$C]-DL-Tyr | 0.005 | | | | |

[a] Samples were dissolved in Bray's solution and counted in a Packard Tricarb liquid scintillation counter. [b] The results, except for barium carbonate, are the average of at least three crystallizations in which the specific activity remained constant (±4%). [c] 10–20 $\mu$Ci of a precursor (5–60 mCi/mmol) was used for each feeding. [d] $NaBH_4$ was added to the counting solution to maintain a colorless solution.