(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-15α 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-15β 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:

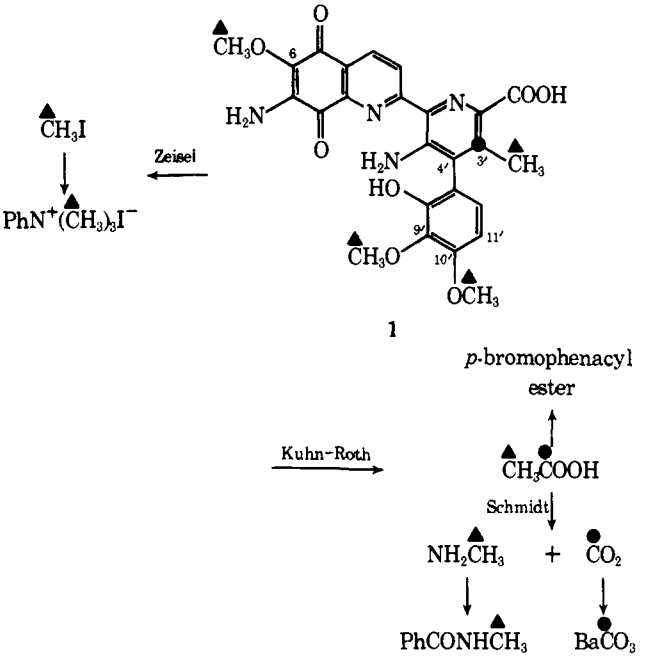
Streptonigrin (**1**), an antibiotic produced by *Streptomyces flocculus* ATCC 13257,[1,2] has striking anticancer properties[3] and is the most potent bone marrow depressant drug known.[4] The structure was originally deduced from chemical studies[5] and was recently confirmed by x-ray crystallography[6] 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_3s (1.1 each). No other single carbon is significantly labeled by methionine.

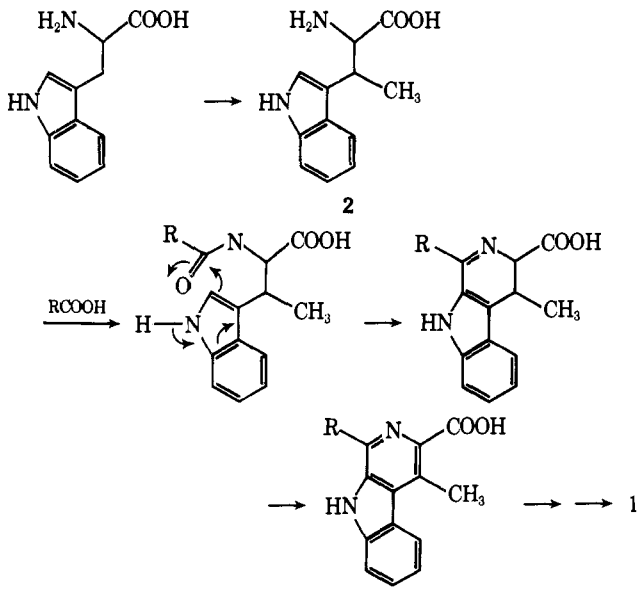
Although only 60–70% of the radioactivity of **1** obtained

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 μCi 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.

Scheme II



from the tryptophan feeding was isolated as barium carbonate, the complete lack of radioactivity in the *N*-methylbenzamide clearly locates the tryptophan label at C-3′. This gave us the opportunity to evaluate the somewhat tentative ^{13}C NMR assignments of the pyridine carbons.[7] [3-^{13}C]-DL-Tryptophan was synthesized,[16] and 82 mg (0.40 mmol, 90% enriched) were fed into eight of the large fermentation flasks. Workup afforded 76 mg of streptonigrin. Only the signal at 137.8 ppm of the ^{13}C NMR spectrum was enhanced.[17] Lown[7] had assigned this to C-4′ and the signal at 135.3 ppm to C-3′.

These results support the biosynthetic pathway shown in Scheme II. Tryptophan is methylated to β-methyltryptophan[15] (**2**) and condenses with a quinolinecarboxylic acid (or precursor thereof). Formation of the pyridine ring then results from the intramolecular attack on an amide carbonyl by the nucleophilic α position of the indole, followed by aromatization of the resulting dihydropyridine and cleavage[18] of the indole ring. This represents a new pathway for the formation of pyridine rings[19] and a new metabolism of tryptophan[20].

It is apparent from our labeling experiments that an intact tryptophan is not incorporated into the quinoline quinone A–B ring system of **1**. This rules out two of the three known pathways to quinoline rings.[21] We are currently investigating the origin of the quinoline quinone portion.

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Steven J. Gould,* Chiu Chin Chang
School of Pharmacy Section of Medicinal Chemistry and Pharmacognosy
University of Connecticut, Storrs, Connecticut 06168
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The Intramolecular Hydrogen Bond in Malonaldehyde as Determined by X-Ray Photoelectron Spectroscopy

Sir:

Recently the intramolecularly H-bonded enol forms of certain 1,3 diketones (**1–3,** Scheme I) have been examined by theoretical[1] and experimental[2] techniques with the goal of deciding whether the enol forms are best represented as an asymmetric C_s structure (**a** or **c**) or a symmetric C_{2v} structure (**b**). We wish to report results concerning O_{1s} binding energies obtained from x-ray photoelectron spectroscopy for compounds **1–3** and for the nonenolized 3,3-dimethylacetylacetone (**4**) which shed light on this fundamental question.

It is expected that the symmetric C_{2v} form (**b**) should show a single ionization from its equivalent oxygens, while the asymmetric form (**a** or **c**) should give rise to two different ionizations which might be resolvable or contribute to a broadened signal. Table I shows the O_{1s} binding energies for malonaldehyde (**1**),[3] acetylacetone (**2**),[6a] hexafluoroacetylacetone (**3**),[6b] and 3,3-dimethylacetylacetone (**4**).[7]

The data for the nonenolizable **4** clearly indicate a single O_{1s} ionization (half-width = 1.77 eV). On the other hand **1,** which is entirely enolized in solution[3] and presumably so in the gas phase,[2a] shows an O_{1s} ionization which deconvolutes into a small peak[8] and two larger ones in a 1:1 area ratio. In the ab-