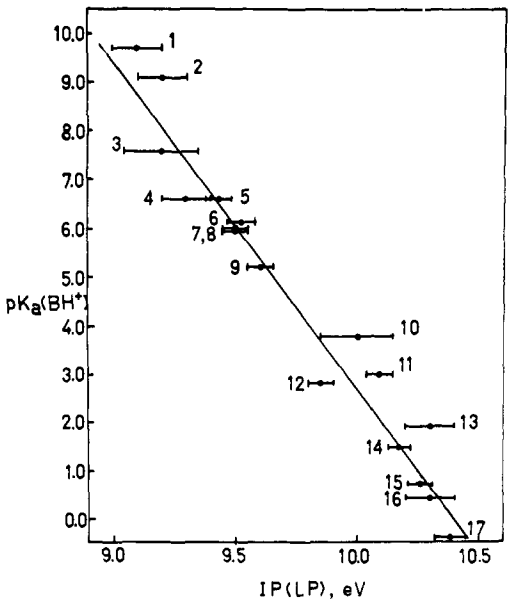

Figure 2. A plot of substituted pyridine pK_a's *vs.* nitrogen lone pair ionization potential: (1) 4-Me_2N, (2) 4-H_2N, (3) 2,4,6-Me_3, (4) 3,4-Me_2, (5) 4-MeO, (6) 3,5-Me_2, (7) 4-Me, (8) 2-Me, (9) H (pyridine), (10) 4-Cl, (11) 3F, (12) 3-Cl, (13) 4-CN, (14) 3-CN, (15) 3,5-Cl_2, (16) 2-Cl, (17) 2-F.

relative proton affinities for 4-NO_2-, 4-CF_3-, 4-H-, 4-CH_3-, and 4-$CH_3OC_5H_9N$ are found to be linear functions of lone pair ionization potentials; PA(R) − PA(H) = −0.3(kcal/eV)IP + 29 kcal.

These results support: (1) the conclusion that a separate order of gas phase and solution phase substituent effects for electron ionization, gas phase proton affinities, and solution phase pK_a's of substituted pyridines is unnecessary, (2) the Heilbronner assignment[3] n, π, π for the pyridine pe IP's, and (3) the proposal that substituent effects on differences in heats of solvation $\delta(\Delta H_5(B:) - \Delta H_5(BH^+))$ of closely related bases and conjugate acids may be linear functions of gas phase proton affinities or lone pair ionization potentials.

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Geldanamycin Biosynthesis and Carbon Magnetic Resonance[1,2]

Sir:

The ansamycin antibiotics—rifamycin, streptovaricin, tolypomycin, geldanamycin, and their derivatives—are of considerable current interest, both for their novel structures and for their biological properties, which include potent inhibition of RNA-dependent DNA polymerase (reverse transcriptase).[3] Biosynthetic results on two of the naphthoquinonoid ansamycins—streptovaricin[2,4] and rifamycin[5]—have recently been reported. We report here our biosynthetic results on geldanamycin,[6] the only benzoquinone representative among the ansamycins, and show that this antibiotic, although structurally different, follows the same biosynthetic pathways as streptovaricin[2,4] and rifamycin.[5]

A number of ^{14}C-labeled precursors (Table I) were fed to fermentation cultures (0.5–1.0 l.) of *Streptomyces hygroscopicus* var. *geldanus* var. *nova* 2 days after inoculation; after a total of 4–5 days' growth the geldanamycin produced was isolated by minor modification of the procedure described earlier.[7] The results in Table I, showing that [methyl-^{14}C]methionine and [carboxy-^{14}C]propionate are incorporated very well, acetate and malonate less well, and formate very little, suggest a biosynthetic pathway like that of streptovaricin and rifamycin, with the *ansa* chain being formed from propionate and acetate; methionine should label the three *O*-methyl groups. Treatment of methionine-labeled geldanamycin (**1**, 3.74 μCi/mmol) with barium hydroxide in water and tetrahydrofuran gave des-*O*-methylgeldanamycin (**2**, 2.52 μCi/mmol), indicating that one-third of the label resided in the 17A-methoxyl carbon.

To identify the other carbons labeled by methionine and propionate, we have employed carbon-13 magnetic resonance (cmr) spectroscopy. Geldanamycin's cmr absorptions were assigned[8] by off-resonance decoupling experiments, standard chemical shift correlations,[9] specific proton decoupling, and comparison to chemical shifts in the cmr spectra of geldanamycin derivatives[6] and model compounds.[8-11]

Experiments with [methyl-^{13}C]methionine and [carboxy-^{13}C]propionate followed the procedures employed with ^{14}C-labeled compounds (above); results are shown in Table I. ^{13}C-Labeled geldanamycin was analyzed mass spectrometrically (flat topped peaks). That from methionine was 81% unlabeled, 10% mono-, 5% di-, and 4% trilabeled, or an average of 11% labeled at

(1) Presented in part at the 166th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1973, ORGN 124.

(2) Paper III in the series "Carbon-13 as a Biosynthetic Tool" [Paper II: B. Milavetz, K. Kakinuma, K. L. Rinehart, Jr., J. P. Rolls, and W. J. Haak, *J. Amer. Chem. Soc.*, **95**, 5793 (1973)].

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Table I. Incorporation of Labeled Precursors into Geldanamycin

Precursor			Geldanamycin isolated			
Compound	Spec Activity (mCi/mmol)	Amount (μmol)	Spec Activity (μCi/mmol)	Isotope dilution	Amount (mg)	% incorp
[Methyl-^{14}C]methionine	42.6	0.86	34.60	1.22×10^3	169.8	22.66
Sodium [carboxy-^{14}C]propionate	54.9	0.73	14.40	3.82×10^3	60.5	3.71
Sodium [carboxy-^{14}C]acetate	52.9	0.28	0.35	1.51×10^5	247.1	0.90
Sodium [carboxy-^{14}C]malonate	40.0	1.00	1.12	3.59×10^4	58.3	0.27
Sodium ^{14}C-formate	41.7	0.96	0.09	4.63×10^5	160.2	0.04
[Methyl-^{13}C]methionine[a]		1333		9.00	70.0	1.16[b]
Sodium [carboxy-^{13}C]propionate[a]		6400		6.00	130.0	0.60[b]

[a] 90% carbon-13. [b] Calculated from enrichment (determined by mass spectrometric analysis) and yield of geldanamycin *vs.* enrichment and amount added of the ^{13}C-labeled precursors.

each of the three (see following) labeled carbons. The cmr spectrum of methionine-labeled geldanamycin clearly showed that only the three methoxyl peaks, at 56.0, 56.5, and 61.0 ppm (relative intensities 1.08:1.08:0.86), were enriched by ^{13}C. The peak at 61.0 ppm is assigned to C-17A, since it was missing in the cmr spectrum of **2**. That at 56.5 ppm is assigned to C-12A from its upfield shift in the spectrum of geldanamycin acetate.[6] That at 56.0 ppm must then be C-6A. The carbamate carbon, C-7A, was not labeled.

Geldanamycin from propionate was 65% unlabeled, 18% mono-, 10% di-, 5% tri-, and 2% tetralabeled, or an average of 15% labeled at each of the four (see following) labeled carbons. The cmr spectrum of propionate-labeled geldanamycin showed four highly enriched peaks at 31.0, 80.6, 131.9, and 169.1 ppm (relative intensities 1.17:0.90:1.14:0.79), and no others. The first three were identified as C-13, C-7, and C-9, respectively, by specific decoupling of the attached protons at δ 1.45, 4.90, and 5.52.[6] The fourth absorption (at 169.1 ppm) was identified as the amide carbonyl (C-1) by its chemical shift, nearly identical with that of the amide carbonyl in the cmr spectrum of streptovaricin D.[2,4] Labeling of C-1, C-7, C-9, and C-13 corresponds perfectly to the pattern for the amide-head[2,4] direction of biosynthesis (Figure 1) but would not agree with an amide-tail direction, while labeling of C-1 and C-13 argues for a continuous sequence of propionate–acetate units from C-14 through C-1. This is the same pattern found for streptovaricin[2,4] and rifamycin.[5] The benzoquinone unit and its attached carbon (C-15 through C-21) were not labeled by propionate or methionine.

The origin of C-15 through C-21 is still under active investigation. However, it seems clear that the remaining C_7N unit of geldanamycin (C-15 through C-21 and the attached nitrogen) corresponds to part of the naphthoquinonoid units in streptovaricin and rifamycin. Although streptovaricin has a methyl group at C-25,[2,4] which corresponds to C-19 of geldanamycin, we have now shown that this methyl group (as well as the methoxyl and methylenedioxy groups) comes from methionine,[12] extending the previous studies,[2,4] which demonstrated that it did not come from propionate.

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(12) B. I. Milavetz, University of Illinois, unpublished results.

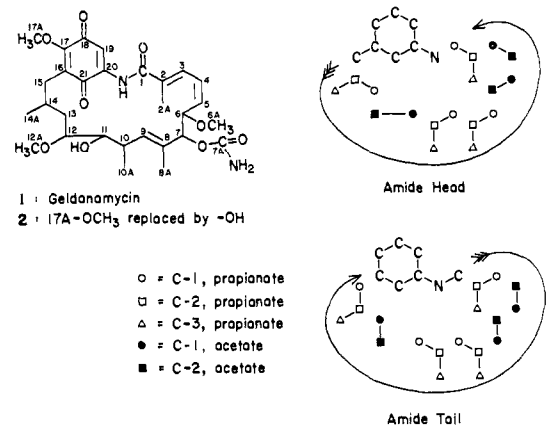

Figure 1. Geldanamycin (**1**), des-*O*-methylgeldanamycin (**2**), and two potential biosynthetic pathways for the formation of **1**. The amide head pathway is correct. Carbons 6A, 12A, and 17A are labeled by methionine.

Supplementary Material Available. A more detailed description of the methods used to assign chemical shifts to specific carbon atoms will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-74-3316.

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Allene Oxide–Cyclopropanone Isomerization. A Low Barrier Pathway on the CNDO/2 Energy Surface

Sir:

There has been considerable interest recently in the cyclopropanone (III) allene oxide (I) isomerization reaction[1-3] and in the postulated intermediate oxyallyl

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