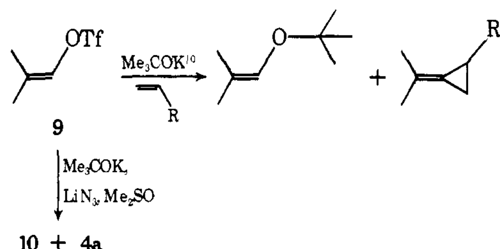
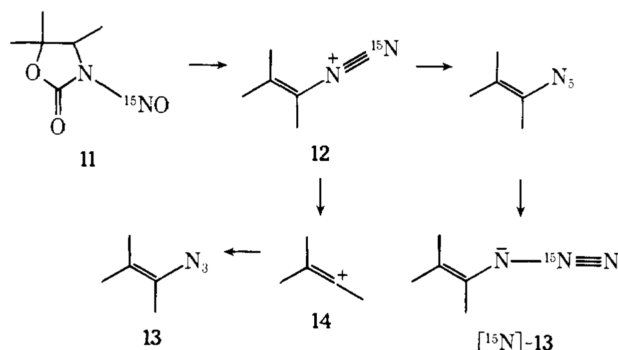
Scheme IV



Scheme V



tassium *tert*-butoxide generates carbene **8** which may be trapped by addition to alkenes.[16]

Additional support comes from a comparison of **1** with 4,5,5-trimethyl-*N*-nitrosooxazolidone (**11**) (Scheme V). Although the yields of vinyl derivatives from trisubstituted nitrosooxazolidones are notoriously low,[5] recovery of a ^{15}N label from [^{15}NO]-**11** in vinyl azide **13** is informative (Table III). The greater stability of vinyl cation **14** (as compared to **5**) promotes loss of nitrogen from diazonium ions **12** and decreases the amount of azo coupling. The fraction of [^{15}N]-**13** is further diminished by addition of lithium perchlorate and, less strongly, by increasing the concentration of lithium azide (salt effect). Addition of lithium methoxide, however, does not affect the retention of ^{15}N (even a slight increase is found) as **12** cannot form a carbene.

We conclude that vinyl azides can arise from vinyldiazonium ions via pentazenes (azo coupling), vinyl cations, and alkylidene carbenes. The relative contributions of these mechanistic pathways may be influenced in a predictable manner by the choice of reaction conditions and by structural variations.

Acknowledgment. We are grateful to Dr. Dietrich Müller for mass spectrometric analyses of the vinyl azides.

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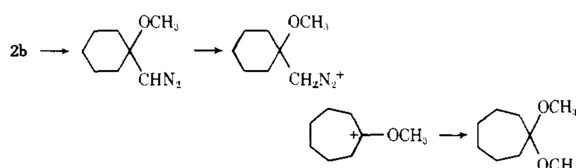
(8) Minor products arise by addition of nucleophiles to the activated double bond of **2**,[5] e.g., cycloheptanone dimethyl acetal was found among the products from **1b**:



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(11) Mass spectrometric analysis (15 eV) of the vinyl azides rests on relative peak intensities of M^+, $(M + 1)^+$, and $(M + 2)^+$. High resolution mass spectrometry resolved the $(M + 1)^+$ peak into [D]-**4** and [^{15}N]-**4** (*m/e* 98.0703 and 98.0610 for **4a**; *m/e* 138.1016 and 138.0923 for **4b**).
(12) Nitrosooxazolidones (**1**) do not undergo H–D exchange prior to decomposition. No deuterium was found, within the limits of NMR detection (<5%), in **1a,b** recovered after ca. 50% conversion.
(13) The lower retention of ^{15}N in **4b** (as compared to **4a**, Table I) may be traced to an increased contribution of the carbene mechanism with **1b** (about twice as much [D]-**4** is produced from **1b**, Table II). In terms of Scheme III this result indicates a higher rate of decomposition vs. protonation of **7b** (ca. 2) as compared to **7a** (ca. 0.5).
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Wolfgang Kirmse,* Otto Schnurr
Abteilung für Chemie der Ruhr-Universität
4630 Bochum, West Germany
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Biosynthetic Origin of the C_2 Units of Geldanamycin and Distribution of Label from D-[6-^{13}C]Glucose[1,2]

Sir:

Considerable interest has been evidenced recently in the biosynthesis of the ansamycin antibiotics, which are both active antibacterial agents and potent inhibitors of reverse trascriptase.[3] The aliphatic ansa ring carbons of rifamycin S,[4] streptovaricin D,[5] and geldanamycin[2] have been shown to be derived mainly from propionate (Figure 1),[6] while the biogenetic C_2 units in the ansa chains of streptovaricin D[5a,b] and rifamycin S[4a,b] have been shown to be derived from acetate or malonate. Recently, White and co-workers[4a] showed that part of the naphthoquinone ring of rifamycin S is derived from acetate (or malonate) and propionate (as is part of the naphthoquinone methide ring of streptovaricin D[5a,b,7]), while the remaining C_7N unit (see Figure 1) is derived from glucose or glycerate by a shikimate-type pathway (Figure 2).[4c,d] In the present communication we present evidence from D-[6-^{13}C]glucose administration which provides remarkable insight into the intermediary metabolism of *Streptomyces hygroscopicus*, demonstrating that 21 of the 29 carbons of geldanamycin arise from C-6 of glucose and, in particular, that the aromatic (benzoquinone) C_7N unit of geldanamycin, like that of rifamycin S (and presumably that of streptovaricin D), arises

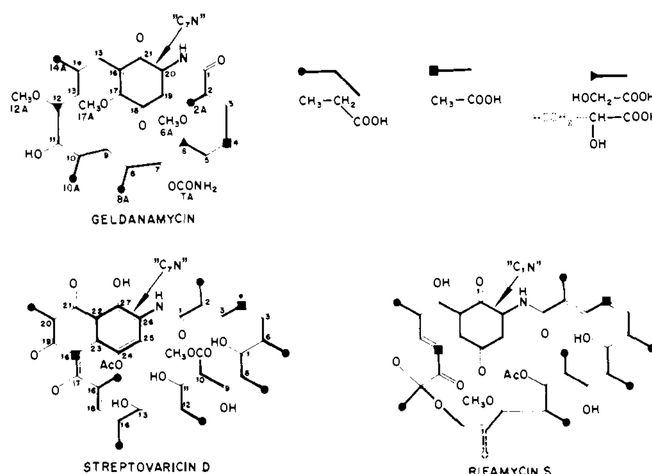

Figure 1. Ansamycin antibiotics and the origin of their carbon skeletons.

Table I. Incorporation of Labeled Precursors into Geldanamycin

Precursor[a,b]				Geldanamycin isolated[a]			
Compound	Sp act, mCi/mmol	Atom % excess	Amount, µmol	Amount, mg	Sp act,[f] µCi/mmol	Isotope dilution[f]	Inc,[f] %
Sodium [*carboxy*-^{14}C]malonate	40.0		0.63	236.0	2.28	1.75×10^4	1.63[g]
D-[6-^{14}C]Glucose	5.06		4.90	104.2	1.31	3.86×10^3	0.89
[U-^{14}C]Shikimic acid	1.9		10.8	49.4	0.150	1.30×10^4	0.02
L-[*guanido*-^{14}C]Arginine	25.9		0.482	64.4	1.66	1.56×10^4	0.84
Sodium D,L-[*carboxy*-^{14}C]glycerate	0.406		36.7	206.1	0.489	8.30×10^2	1.05[h]
Calcium [*carboxy*-^{14}C]glycolate	55.0		0.321	89.3	1.06	5.19×10^4	0.85
Calcium [*methylene*-^{14}C]glycolate	55.0		0.317	66.2	1.26	4.36×10^4	0.79
Sodium [*methylene*-^{13}C]malonate[c]		91	3910	110.4		21.0	0.24[i]
Calcium D,L-[*carboxy*-^{13}C]glycerate[d]		87	5940	57.1		28.3	0.15[h,i]
Calcium [*carboxy*-^{13}C]glycolate		85	1970	91.8		69.1	0.30[i]
D-[6-^{13}C]Glucose[e]		40	13850	109.9		13.7	2.16[i]
L-[*guanido*-$^{15}N_2$,^{13}C]Arginine hydrochloride		92 (^{13}C) 94 ($^{15}N_2$)	569	92.0		26.4	1.09[i]

[a] Precursors were added to the production media 2.0 days after inoculation, and the geldanamycin was isolated 2.0–2.5 days later by chromatography of the chloroform extracts of the filtered broths. [b] All precursors were administered to 400–500 ml of production medium[9] containing 40 mg/mL of glucose, except as noted: [c] 1100 mL of production medium; [d] 300 mL of production medium; [e] 15 mg/mL of glucose. [f] The specific activities listed are the final values obtained for recrystallized antibiotic, and the percent incorporation is based on the initial specific activity of the isolated geldanamycin. [g] No allowance has been made for loss of half of the radioactivity as $^{14}CO_2$. [h] Incorporation is based on the theoretical utilization of both enantiomers. [i] Incorporation and dilution were determined from the ^{13}C NMR data.

Table II. Chemical Shift Assignments andive Enrichments[10] of Carbons of Geldanamycin Labeled by D-[6-^{13}C]Glucose, Calcium D,L-[*carboxy*-^{13}C]Glycerate, and Calcium [*carboxy*-^{13}C]glycolate

Carbon atom	Chemical shift	Relative enrichment		
		D-[6-^{13}C]Glucose	Calcium D,L-[*carboxy*-^{13}C]glycerate	Calcium [*carboxy*-^{13}C]glycolate
1	169.1	2.50	1.00	1.04
2	133.2*	4.33	0.92	1.02
2A	12.2//	4.18	1.03	0.98
3	128.4	1.51	1.06	1.00
4	125.7	5.25	0.98	1.04
5	137.8	0.95	4.57	2.40
6	81.6	1.36	1.13	1.04
6A	56.0†	3.24	1.06	1.00
7	80.6	2.51	1.09	1.08
7A	156.0	1.99	2.67	2.18
8	132.6*	4.08	0.95	1.01
8A	12.5//	4.34	1.01	1.06
9	131.9	2.83	1.09	1.26
10	32.1	4.04	1.32	1.11
10A	23.3≠	4.26	0.96	0.99
11	71.9	1.00	4.92	2.37
12	80.2	1.34	0.98	1.10
12A	56.5†	3.12	1.04	1.06
13	31.0	2.85	1.49	1.15
14	26.6	4.17	0.99	1.06
14A	13.0≠	3.89	0.89	0.96
15	31.7	1.57	4.60	2.12
16	128.1	1.13	1.02	0.93
17	156.4	7.01	1.03	1.01
17A	61.0	2.97	1.06	0.97
18	183.6	1.05	0.95	0.86
19	110.9	0.93	2.23	1.50
20	139.6	0.76	0.99	1.00
21	183.1	6.01	1.12	0.98

[a] Chemical shifts are given in parts per million downfield from internal Me_4Si in Me_2SO-d_6 solution. Signals marked with the same superscript may be interchanged.

from glucose by a shikimate-type pathway. We also present evidence from malonate, glycerate, and glycolate administrations which shows that geldanamycin biosynthesis represents a variation on the previously accepted ansamycin scheme, since, in contradistinction to rifamycin S and streptovaricin D biosynthesis, sodium [*methylene*-^{13}C]malonate labels only one of the three C_2 units in geldanamycin, while both calcium [*carboxy*-^{13}C]glycerate and calcium [*carboxy*-^{13}C]glycolate label the two other C_2 units. Finally, we present data which suggest a possible origin of the C_1N (carbamate) unit (see Figure 1).

Although sodium [*carboxy*-^{14}C]acetate added to fermentation cultures of *Streptomyces hydroscopicus* var. *geldanus* var. *nova* labeled geldanamycin (0.90% incorporation),[2] we have been able to detect no labeling of geldanamycin by either mass spectrometry or ^{13}C NMR spectroscopy, when sodium

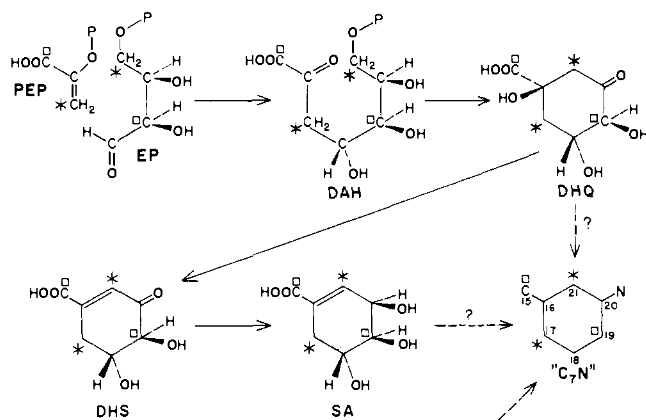

Figure 2. Conversion of phosphoenolpyruvic acid (PEP) and erythrose 4-phosphate (EP) to possible precursors (DAH = 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate, DHQ = 3-dehydroquinic acid, DHS = 3-dehydroshikimic acid, SA = shikimic acid) of the "C_7N" unit common to geldanamycin, streptovaricin D, rifamycin S, and other antibiotics. The numbers on the "C_7N" unit refer to positions in geldanamycin. D-[6-^{13}C]Glucose labels to a high degree those carbons indicated by an asterisk (*). Calcium D,L-[*carboxy*-^{13}C]glycerate and calcium [*carboxy*-^{13}C] glycolate label those carbons indicated by a square (□).

[*carboxy*-^{13}C]acetate (90 atom % ^{13}C) was administered at a level of 0.20 mg/mL.[8] However, sodium [*carboxy*-^{14}C] malonate was incorporated even better (1.63%) than acetate into geldanamycin (Table I), and administration of sodium [*methylene*-^{13}C]malonate[5b] (91 atom % ^{13}C) to fermentation cultures[9] at a level of 0.53 mg/mL afforded labeled geldanamycin. Label, however, was found only at C-4 (see Figure 1 for numbering scheme), to the extent of 4.95 times natural abundance,[10] an unexpected result, since from the streptovaricin D and rifamycin S results we anticipated C-6 and C-12 would be labeled also. It appeared to us to be significant that these unlabeled carbons (C-6, C-12) in the other two C_2 units (C-5, C-6; C-11, C-12) are oxygenated, and we have confirmed the apparent significance by demonstrating that both of these C_2 units in geldanamycin are derived from glycerate or glycolate (vide infra).[11]

[*carboxy*-^{14}C]Glycerate[12] and [*carboxy*-^{14}C]- and [*methylene*-^{14}C]glycolate labeled geldanamycin well (Table I), the approximately equal incorporation obtained from the latter two precursors suggesting that the two carbons of glycolate remain intact. Administration of calcium D,L-[*carboxy*-^{13}C]glycerate[12] (87 atom % ^{13}C) to fermentation cultures at a level of 5.7 mg/mL afforded antibiotic labeled at C-5 and C-11 to the extent of about 4.7 times natural abundance (Table II).[10] A possible route from glycerate to geldanamycin might involve the incorporation of intact glycerate into the growing ansa chain followed by oxidation of the hydroxymethyl carbon and decarboxylation. An alternative explanation would involve its conversion to glycolate (perhaps via glyoxylate)[13a,b] and incorporation of glycolate. Incorporation of either glycerate or glycolate would involve a novel variation of the acetate–propionate pathway.[14] Support for the glycerate → glycolate possibility is provided by the labeling pattern observed upon administration of calcium [*carboxy*-^{13}C]glycolate[15] (85 atom % ^{13}C, 0.94 mg/mL), in which the same carbons in the ansa chain are labeled as in the glycerate feeding (Table II).[10,16]

L-[*guanido*-^{14}C]Arginine labeled geldanamycin (Table I), and administration of L-[*guanido*-$^{15}N_2$,^{13}C]arginine hydrochloride[17] (0.30 mg/mL, 92 atom % ^{13}C, 94 atom % $^{15}N_2$, $J_{^{15}N-^{13}C}$ = 21.3 Hz) afforded geldanamycin labeled at C-7A only, to the extent of 4.16 times natural abundance. We detected no dilution of the ^{15}N label by ^{13}C NMR spectroscopy and observed a ^{15}N–^{13}C coupling constant of 26.5 Hz in the antibiotic. The carbamate residue in geldanamycin may be derived from the guanido group of arginine directly, from the ureido group of citrulline, or from carbamyl phosphate formed in the arginine dihydrolase pathway.[18] Similar results were obtained for the carbamate unit in mitomycin.[19]

D-[6-^{14}C]Glucose was incorporated well (Table I) into geldanamycin, and administration of 2.5 g of D-[6-^{13}C]glucose[20] (40 atom % ^{13}C) to 400 mL of production medium (containing 15 mg/mL of glucose rather than the normal 40 mg/mL[9]) after 2 days of fermentation afforded 109.9 mg of geldanamycin, labeled in all but eight carbons (Table II).[10] ^{13}C-Enriched carbons include C-15, C-17, and C-21, in addition to those carbons derived from propionate,[2] malonate, arginine, and methionine[2] (vide infra).

The most highly enriched carbons of the antibiotic are C-17 and C-21. That these carbons are labeled is readily explained by reference to the shikimate pathway in Figure 2,[21] although our data do not allow us to determine whether C-15, C-16, and C-17 or C-15, C-16, and C-21 are derived from phosphoenol pyruvate.[4d] The high enrichments of C-17 and C-21 are indicative of an efficient conversion of D-[6-^{13}C]glucose to [3-^{13}C]phosphoenolpyruvate and [4-^{13}C]erythrose 4-phosphate and, presumably, to labeled 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate.[21] Since shikimic acid is not incorporated well into geldanamycin (Table I), it may be that 3-dehydroquinic acid, 3-dehydroshikimic acid, or another closely related intermediate (such as the recently isolated and characterized *Streptomyces* metabolites 2-hydroxymethyl-4,5,6-trihydroxy-2-cyclohexenone[22a] and 1-carboxy-5-amino-1,3-cyclohexadiene[22b]) is more directly involved than shikimate in the formation of the quinone ring; alternatively, shikimate may be transported poorly through the cell wall.

The remaining labeling pattern is readily explained by well known pathways of intermediary metabolism. D-[6-^{13}C] Glucose is efficiently converted via pyruvate to [*methyl*-^{13}C]acetate by glycolysis,[13b] so that carboxylation of acetate to form [*methylene*-^{13}C]malonate (vide supra)[23] explains the relatively high enrichment (5.25 times natural abundance) of C-4. Entry of the [*methyl*-^{13}C]acetate into the tricarboxylic acid (TCA) cycle[13c] forms [*methylene*-^{13}C]succinate, which is converted by methylmalonylmutase[13b] to [α-^{13}C]- and [*methyl*-^{13}C]methylmalonates and the equivalent [*methylene*-^{13}C]- and [*methyl*-^{13}C]propionates. The indistinguishability of the methylene carbons of succinate accounts for the approximately equal distribution of label (3.9–4.3 times natural abundance) at C-2 and C-2A, C-8 and C-8A, C-10 and C-10A, and C-14 and C-14A of geldanamycin, while their lower enrichment than that at C-4 is due to the greater number of conversions the label has undergone.

After a second turn of the TCA cycle [*carboxy*-^{13}C]methylmalonate (the equivalent of [*carboxy*-^{13}C]propionate) is formed by the above mechanism.[13b,c] Thus, the enrichments at C-1, C-7, C-9, and C-13 are approximately the same (2.5–2.8 times natural abundance), but lower than the enrichments of the eight previously noted propionate-derived carbons. Also, after two turns of the TCA cycle oxaloacetate becomes labeled at each carbon, and its conversion to [*carboxy*-^{13}C]pyruvate[13b] and [*carboxy*-^{13}C]acetate[13c] (via [α-^{13}C]pyruvate) accounts for the small amount of label (ca. 1.5 times natural abundance) at C-15 and C-3, respectively. Furthermore, conversion of [^{13}C]carbon dioxide formed in the TCA cycle to [^{13}C]carbamyl phosphate and then, perhaps, to [*guanido*-^{13}C]arginine by the urea cycle[13d] explains the labeling (2.0 times natural abundance) at C-7A (vide supra).

Finally, the methoxyl carbons can be labeled (ca. 3.0 times natural abundance) by [*methyl*-^{13}C]methionine[2] formed from D-[6-^{13}C]glucose via D-3-phospho[3-^{13}C]glycerate, [3-^{13}C]serine, and N^5-[*methyl*-^{13}C]methyltetrahydrofolate.[13c]

Our results, coupled with those of the White group,[4] suggest

a common origin for the C_7N unit of other natural products,[24] and it has been proposed that the C_7N unit in mitomycin C is also derived from glucose by a shikimate-type pathway.[25] On the other hand, the different sources of the C_2 units in geldanamycin vis-a-vis streptovaricin and rifamycin provide an interesting biosynthetic variation for molecules which appear to be otherwise biogenetically very similar.

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(7) The "extra" aromatic methyl group in streptovaricin D is derived from methionine.[5b]
(8) Increasing the concentration of exogenous acetate decreases the yield of antibiotic.
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(16) D,L-[*carboxy*-^{13}C]Glycerate and [*carboxy*-^{13}C]glycolate also label C-7A, C-15, and C-19 (Table II). Labeling of these three carbons can be explained by arguments similar to those provided for the distribution of label from D-[6-^{13}C]glucose (see text).
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Arthur Haber,[26] Ronald D. Johnson, Kenneth L. Rinehart, Jr.*
Roger Adams Laboratory, University of Illinois
Urbana, Illinois 61801
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Charge Distribution in Large Polyoxoanions: Determination of Protonation Sites in $V_{10}O_{28}^{6-}$ by ^{17}O Nuclear Magnetic Resonance

Sir:

Several polyoxoanions of the early transition elements are known to be protonated in solution,[1-3] and/or the solid state.[3-6]

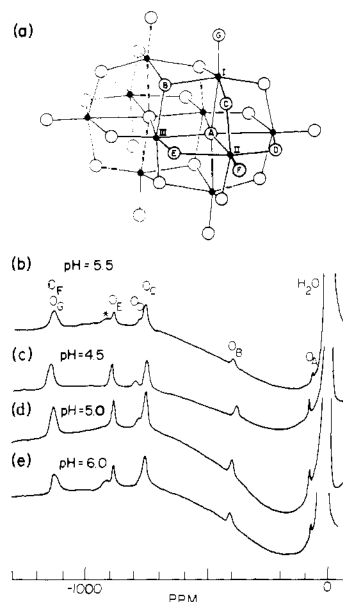

Figure 1. The D_{2h} symmetized structure of $V_{10}O_{28}^{6-}$ (see ref 13, 14) is shown in (a). Small filled circles represent vanadium atoms and large open circles represent oxygen atoms. One member of each symmetry equivalent set of atoms is labeled. ^{17}O FTNMR spectra of $V_{10}O_{28}^{6-}$ in H_2O are shown in b–e. All spectra were measured at 25 °C, with a total vanadium concentrations of 1.5–1.8 M. Chemical shift assignments are given in (b), where the asterisk labels a metavanadate resonance. For chemical shift data, see Table I.