factors of $10^{6.6}$ and 10^{12}, respectively.[16-21] Thus it seems awkward to interpret it as the **1** → **5** ring inversion rate constant k_1 as required by the originally proposed mechanism. The $A = 2.5 \times 10^{-4}$ sec^{-1} value is, however, entirely reasonable in terms of the alternative in which $A = Kk_1$. The constant K might well be 10^{-2} or 10^{-3}, and E_a for the conversion **7** → **8** could be on the order of 20 kcal/mol.[22]

In terms of this alternative and kinetically plausible mechanism, the stereochemistry of adducts from methyl-substituted analogs of **1** would depend on the relative rates of isomerization **7** → **8** in two distinct conrotatory modes.[3] Further work on the conformational, valence isomerization, and cycloaddition chemistry of triene **1** will be required to test this deduction.

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D-Glucosamine and L-Citrulline, Precursors in Mitomycin Biosynthesis by *Streptomyces verticillatus*

Sir:

The mitomycins (I, mitomycin B; II, mitomycin C) are a group of anticancer antibiotics which contain a unique carbon–nitrogen ring skeleton[1] and which are produced by *Streptomyces verticillatus* and other strains of *Streptomyces*.[2] Previous studies on their biosynthesis have shown that L-[methyl-^{14}C]methionine provides *O*- and *N*-methyl groups but not the *C*-methyl group,[3-5] that L-[guanidino-^{14}C]arginine labels the carbamoyl group,[4] that label from D-glucose[5] and from D-ribose[4] appears in the methylbenzoquinone moiety, and that D-[1-^{14}C,6-^{3}H,^{15}N]glucosamine is incorporated in a manner suggesting its utilization as an intact unit.[4,6]

To further examine the intact incorporation of this amino sugar, D-[1-^{13}C,^{15}N]glucosamine was prepared from D-arabinose, [^{15}N]benzylamine (99 atom % ^{15}N), and H^{13}CN (90 atom % ^{13}C).[7] Fifty milligrams of this material was mixed with 5 μCi of D-[1-^{14}C]glucosamine administered to doubly replaced mycelia of *S. verticillatus* and approximately 4 mg of a mixture of mitomycins A, B, and C and porfiromycin were isolated 24 hr later. Carbon-14 incorporation[8] into this mixture was 1.8%. Mitomycin B was purified and analyzed by mass spectrometry as described previously.[6] The relevant ions of the cluster C_4H_8N (*m/e* 70), which according to Van Lear[9] comprise C-1, C-2, and C-3, N-1a, and its attached methyl group, were identified in high-resolution mass spectra (CEC 21 110B, direct inlet probe, 200°, 70 eV, mass marker: perfluorokerosene at *m/e* 69.99856; accuracy, 3 mmass units). Their relative intensities (Table I) were determined using an average of ten scans per ion. It can be calculated from the intensity data that the specific incorporation of ^{13}C into the C_4H_8N fragment, most likely into its C-3, was 36.9%, while the specific incorporation of ^{14}C[10] into mitomycin B was 41.2%. This close agreement, the small value observed for ion c, and the virtual absence of ion e in the spectrum show that only a negligible fraction of the carbon label is randomized and indicate that the incorporation is specific. The amino group of D-glucosamine apparently provides directly the nitrogen atom of the aziridine ring, yet the configuration at C-2 of the mitomycins[11] is opposite to that at C-2 of this aminohexose. Since the intensities of ions b and c were very weak the ^{13}C and the ^{15}N labels are never separated, and it can be concluded that the nitrogen atom is not removed from the carbon skeleton and reincorporated during the inversion of the configuration.

Table I. Relative Abundance of Ions Belonging to the Ion Cluster C_4H_8N of Mitomycin B Isolated upon Feeding D-[1-^{13}C,^{15}N]Glucosamine to *S. verticillatus*

Ion	Designation	Rel abundance × 100
C_4H_8N	a	55.6
$C_4H_8{}^{15}N$	b	8.4
$C_3{}^{13}CH_8N$	c	4.9
$C_3{}^{13}CH_8{}^{15}N$	d	31.1
$C_2{}^{13}C_2H_8N$	e	<2

In another feeding experiment D-[6-^{14}C]glucosamine (5 μCi, 5 mg) which was synthesized from D-[6-^{14}C]-gluconic acid *via* D-[5-^{14}C]arabinose[7] gave 5.1% incorporation into mitomycins A, B, and C and porfiromycin. Mitomycin C after purification to constant specific radioactivity (first recrystallization, 9.37×10^4 dpm/mmol; second recrystallization, 9.34×10^4 dpm/mmol) was converted into 2-amino-1,7-dihydroxydecarbamoylmitosene (III).[12,13] This compound was subjected to periodate oxidation to give formaldehyde, which arises predominantly from C-10.[14] The latter was purified

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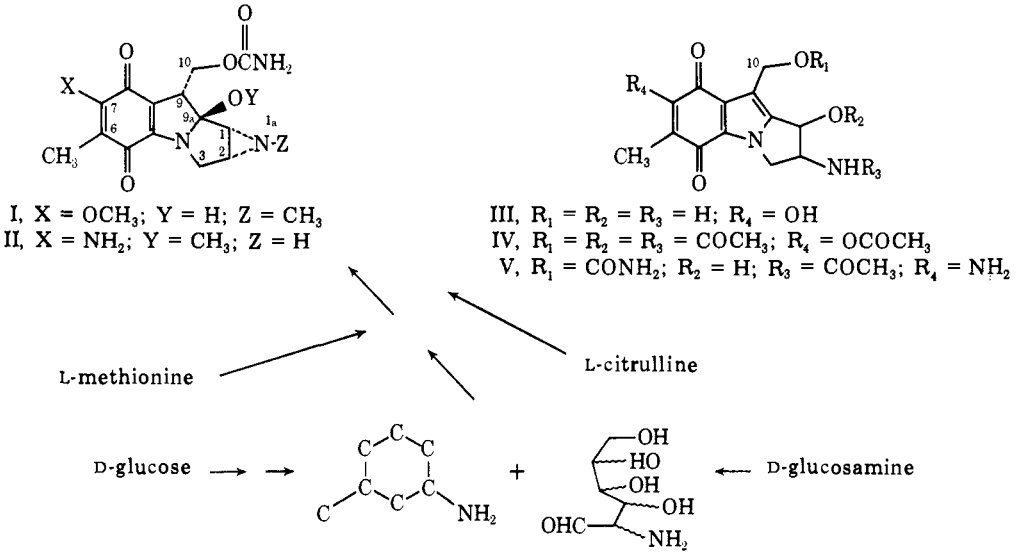
Scheme I. Structures of Mitomycins B and C, of Degradation Products, and of Intermediates in a Hypothetical Biogenetic Scheme



as the dimedone derivative (first recrystallization, 6.93×10^4 dpm/mmol; second recrystallization, 6.72×10^4 dpm/mmol) and was found to have 72% of the specific radioactivity of the starting mitomycin C. It thus appears that C-6 of D-glucosamine is incorporated predominantly or exclusively into C-10 of mitomycin C.

Based on the above results, and on previous studies which demonstrated that D-[1-^{14}C,6-^{3}H]glucosamine is incorporated into mitomycins with 91% tritium retention,[4] we conclude that C-1, -2, -3, -9, -9a, and -10 and the nitrogen atom of the aziridine ring of the mitomycins are derived from the intact carbon–nitrogen skeleton of D-glucosamine or another hexosamine arising from it.

L-[ureido-^{14}C]Citrulline was considered to be a possible intermediate in the conversion of the amidino group of L-[guanidino-^{14}C]arginine into the carbamoyl group of the mitomycins. Competition feeding experiments were carried out with these amino acids to investigate which is the more proximate precursor. The results (Table II) show that while the uptake of these amino acids into the mycelium is of a comparable magnitude, L-citrulline is more readily incorporated into the mitomycins. The label from L-[ureido-^{14}C]-citrulline is found predominantly in the carbamoyl group as shown by degradation of the resulting mitomycin C (sp act., 1.1×10^6 dpm/mmol) to give mitosene IV[16] which carried no radioactivity and mitosene V[16] (sp act., 8.5×10^5 dpm/mmol) which retained 77% of the specific radioactivity of the antibiotic. The partial loss of radioactivity in the conversion II → V may indicate some incorporation of label from citrulline into the *O*-methyl group of mitomycin C.

While the data on the incorporation of D-glucosamine and L-citrulline suggest their role as specific precursors for the right-hand portion of the mitomycins, the detailed biogenetic origin of the remaining C-7 unit including the "indolic nitrogen" remains uncertain. It appears that the mitomycins are not the only antibiotics which contain a C-7 unit characterized by a 1,3 arrangement of a one carbon side chain and an amino function on a six-membered ring. Clearly the antibiotics validamycin A[17] and kinamycin C[18] contain similar C-7 units and such units also appear to exist as biosynthetic entities of unknown origin in the antibiotics rifamycin S[19] and geldanamycin.[20,21] We wish to suggest that these antibiotics share with mitomycin C a common biogenetic origin of their C-7 units from compounds arising from carbohydrate metabolism.

Table II. Incorporation of L-Citrulline and L-Arginine into the Mycelium and into Mitomycins in Competition Feeding Experiments with *S. verticillatus*

Precursors added	Radioact. added, μCi	Incorporation into Mycelium	Incorporation into Mitomycins
9 μmol of L-[guanidino-^{14}C]-arginine + 26 μmol of L-citrulline	5.7	33.5	5.8
36 μmol of L-[guanidino-^{14}C]-arginine (9 μmol labeled, 27 μmol nonlabeled)	5.7	31.4	5.7
36 μmol of L-[ureido-^{14}C]-citrulline (9 μmol labeled, 27 μmol nonlabeled)	4.7	24.3	8.2
9 μmol of L-[ureido-^{14}C]-citrulline + 26 μmol of L-arginine	4.7	29.0	10.4

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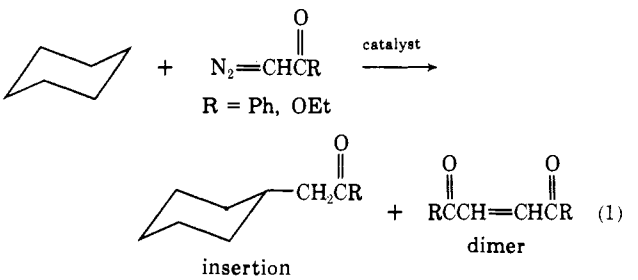
Intermolecular Carbon–Hydrogen Insertion of Copper Carbenoids

Sir:

Free carbenes, generated by the photolysis or thermolysis of aliphatic diazo compounds, have enjoyed little popularity in the cyclopropanation of olefins because insertion of the reactive intermediate into carbon–hydrogen bonds often competes effectively with olefin addition.[1] Catalysis of the decomposition by copper metal or its salts generally suppresses C–H insertion,[2] however, except in favorable intramolecular cases,[3,4] and this observation has led to the widely held tenet[2] that copper carbenoids lack the necessary reactivity to insert into C–H bonds. It has never been clearly established whether carbenoids or adventitious free carbenes account for the C–H insertion by-products occasionally reported[5,6] from the decomposition of diazo compounds in the presence of copper catalysts; consequently, the only authenticated case of intermolecular copper carbenoid C–H insertion to date appears to be that of CH_2 into the relatively special carbon–hydrogen bonds of hexamethyldisilane and tetramethylsilane[7] (5–10% yield). We have now demonstrated by the experiments described below that certain copper carbenoids, when deprived of alternative reaction pathways, can and do insert intermolecularly into unactivated, aliphatic C–H bonds.

Cyclohexane, a solvent often employed for carbenoid reactions[4,8] and commonly regarded as inert, was chosen as the substrate for our studies to ensure that C–H insertion would produce only a single, easily identifiable product (eq 1). High dilution techniques were used to minimize the formation of "dimers" arising from attack of the carbenoid on excess diazo compound. Thus a dilute solution of the diazo compound in 20 ml of dry cyclohexane was added dropwise with vigorous stirring to a refluxing suspension of the catalyst in 100 ml of cyclohexane over a standard period of time. In the absence of catalyst, the diazo compounds remain >90% unchanged, and glc indicates



no more than a trace of the insertion product. With cupric sulfate or cuprous chloride catalysis, however, none of the diazo compound survives, and the C–H insertion product appears in 9–24% yield together with varying amounts of dimer (see Table I).

Table I

Starting materials		% products		
Diazo	Catalyst	Diazo[c]	Insertion[d]	Dimer[e]
N_2=CHCO$_2$Et[a]	None	98	<1	0
	$CuSO_4$	0	24	40
	CuCl	0	15	61
N_2=CHCPh[b]	None	92	0	0
	$CuSO_4$	0	17	9
	CuCl	0	9	29

[a] The reactions of ethyl diazoacetate were run with 4.4 mmol each of catalyst and diazoester for 1 hr as described in the text. [b] The reactions of diazoacetophenone were run with 2.2 mmol each of catalyst and diazoketone for 0.5 hr as described in the text. [c] The amount of recovered ethyl diazoacetate was determined by quantitative uv; recovered diazoacetophenone was determined by nmr with an internal standard. [d] The insertion products were purified by preparative glc and identified by nmr, ir, and mass spectroscopy. Yields were determined by glc with an internal standard, making the appropriate adjustments for detector response factors. [e] Yields of diethyl maleate and diethyl fumarate were determined by quantitative nmr; dibenzoylethylene was analyzed by quantitative glc.

The high recovery of diazo compound from the uncatalyzed control reactions demands that the significant amounts of C–H insertion products formed in the catalyzed reactions be copper–carbenoid derived and not a consequence of thermally generated, free carbenes. Accordingly, the characteristic suppression of C–H insertion normally associated with copper catalysis seems to reflect an enhanced selectivity of the reactive intermediate rather than an incapability of the carbenoid to participate in otherwise competitive processes. It should be noted in this connection that copper catalysis normally suppresses also the Wolff rearrangement of ketocarbenes generated by diazoketone decomposition[2] but that exceptions have been found.[9]

Although glc revealed no other products in our experiments, the poor material balance suggests polymer formation in spite of the high dilution. At even higher dilution with more catalyst the yield of insertion product increases slightly at the expense of dimer formation; the material balance fails to improve, however. Both glc and nmr comparision of the crude reaction

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