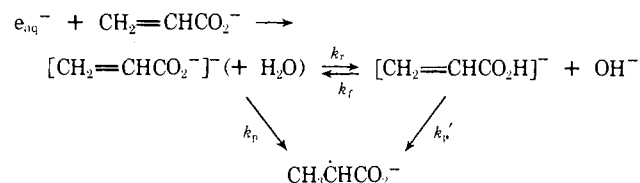constant at pH 10 of 1.5×10^5 sec^{-1} or a half-life of 5 μsec. Below this pH the half-life increases to a maximum of 10 μsec. Thus at the time of the first valid data (3 μsec after the pulse) more than half of the initial conductance change should still be present. Similar curves were obtained at pH 9.9 and 10.9 with 1 mM acrylic acid.

The validity of the conductivity data can be demonstrated by an experiment on acrylamide. Hayon, et al.,[10] report the pK for

$$[CH_2\!\!=\!\!CH\dot{C}(OH)NH_2] \rightleftharpoons CH_2\!\!=\!\!CH\dot{C}(O^-)NH_2 + H^+$$
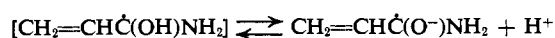
to be 7.9 and the rate constant for protonation on carbon of $CH_2CH\dot{C}(O^-)NH_2$ to be 1.5×10^5 sec^{-1} (half-life of 4.6 μsec). Again, because the $e_{aq}{}^-$ adduct is dissociated above pH 9, there should be a large initial decrease in conductivity followed by a decay back to little or no overall change as the species $CH_3\dot{C}HCONH_2$ is produced. The experimental result shown in Figure 1b is in complete agreement with this expectation. The value at 3 μsec after the pulse corresponds to a conductance change of -60 units. A first-order plot of the points gave a reasonable straight line and allowed extrapolation to the end of the pulse. Average data for five curves gave an initial conductance change of -130 units and a half-life of 4.2 μsec ($k = 1.6 \times 10^5$ sec^{-1}). In this case the $e_{aq}{}^-$ adduct is obviously dissociated and the rate of protonation agrees with previous data.[10]

The conductivity results on the acrylate system together with the qualitatively different behavior of acrylamide show clearly that the $e_{aq}{}^-$ adduct in the former case initially takes up a proton to form $[CH_2\!\!=\!\!CHCO_2H]^-$. The complete reaction scheme for pH >8 can be written as

$$e_{aq}{}^- + CH_2\!\!=\!\!CHCO_2{}^- \longrightarrow$$

$$[CH_2\!\!=\!\!CHCO_2{}^-]^- (+ H_2O) \underset{k_f}{\overset{k_r}{\rightleftharpoons}} [CH_2\!\!=\!\!CHCO_2H]^- + OH^-$$

$$\underset{k_p}{\searrow} \qquad \underset{k_{p'}}{\swarrow}$$

$$CH_3\dot{C}HCO_2{}^-$$

A value for $k_p > 7 \times 10^6$ sec^{-1} can be derived from the immediate appearance of $CH_3\dot{C}HCO_2{}^-$ at pH 12;[1,3] the rate constant k_p' is given as 7.7×10^4 sec^{-1} by Hayon, et al.[1] The rate constant k_r for protonation on the carboxyl group must be larger than k_p because $[CH_2\!\!=\!\!CHCO_2H]^-$ is mainly produced at lower pH. Thus we can take $k_r > 7 \times 10^7$ sec^{-1} (i.e., ten times k_p). The rate constant k_f can reasonably be taken to be[11] 5×10^9 M^{-1} sec^{-1} so that, using k_f and k_r, the pK_a for dissociation of $[CH_2\!\!=\!\!CHCO_2H]^-$ can be estimated to be >12.1. A comparable value can be obtained by considering the linearity of the overall protonation rate with $[OH^-]$ as given in Figure 1 of ref 1. With rate constants as given here, the dynamics of equilibrium 1 are such that the reaction corresponding to k_f will not be rate determining at any pH studied (pH <11.3[1]) and the equilibrium will be maintained. A pK near 12 makes the ion $[CH_2\!\!=\!\!CHCO_2H]^-$ rather similar to $[C_6H_5CO_2H]^-$ which has a pK of 12.0.[12] Parallel
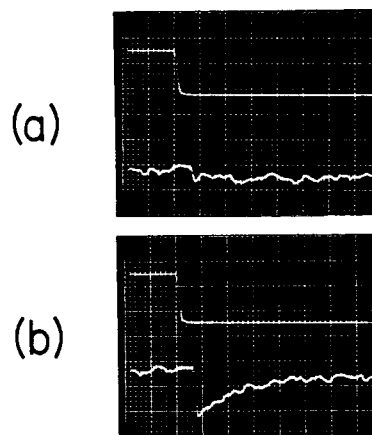

Figure 1. Oscilloscope traces showing the conductivity change following pulse irradiation of (a) 0.5 mM acrylic acid at pH 9.3 and (b) 1 mM acrylamide at pH 9.7. The step in the upper trace of each photograph gives the dose as monitored by a secondary emission chamber; the lower trace in each shows the conductivity change. The dose corresponds to about 1×10^{-5} M of total radicals. All traces have the same sweep rate, 5 μsec per large (cm) division, and start at the same time. The conductivity and dose curves have the same vertical sensitivities in both photographs. The conductivity curve becomes valid at about 3 μsec after the end of the pulse (i.e., at 2.8 cm).[9]

behavior for derivatives of ethylene and benzene is seen also in the pK values of $CH_2\!\!=\!\!CH\dot{C}(OH)NH_2$ (7.9)[10] and $C_6H_5\dot{C}(OH)NH_2$ (7.7).[13]

While the conductivity results suggest a simple mechanism for the catalysis of protonation by OH$^-$, the fact that equilibrium 1 is maintained during reaction rules out any similar mechanism for catalysis by other bases as was reported.[1] Although a shift of equilibrium 1 by high ionic strength would influence the protonation rate, some other process must probably be invoked to explain the increased rate in the presence of the various buffer species.

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Biosynthetic Incorporation of [1-¹³C]Glucosamine and [6-¹³C]Glucose into Neomycin[1,2]

Sir:

The biosynthesis of the commercially important antibiotic neomycin (B = 1, C = 2) has been studied for

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(2) Paper IV in the series "Carbon-13 as a Biosynthetic Tool" (Paper III: R. D. Johnson, A. Haber, B. I. Milavetz, and K. L. Rinehart, Jr., submitted for publication) and Paper XVIII in the series "Chemistry and Biochemistry of the Neomycins" (Paper XVII: W. T. Shier, S. Ogawa, M. Hichens, and K. L. Rinehart, Jr., J. Antibiot., 26, 551 (1973)).

Table I. Enrichment of Carbons in Hexa-*N*-acetylneomycin B (3) from ^{13}C-Labeled Precursors

Carbon[a]	δ, ppm[b]	Enrichment[c] from [1-13C]-Glucosamine	[6-13C]-Glucose	Carbon[a]	δ, ppm[b]	Enrichment[c] from [1-13C]-Glucosamine	[6-13C]-Glucose
C-1	97.0	7.07	0.78	R-1	109.3	1.60	0.83
C-2	54.2	0.86	1.00	R-2	74.5	0.98	0.92
C-3	71.4	1.25	1.30	R-3	77.2	0.94	0.87
C-4	71.4	1.25	1.30	R-4	82.4	0.99	0 88
C-5	71.4	1.25	1.30	R-5	62.2	1.03	2.28
C-6	40.8	0.74	1.94	B-1	98.5	6.32	0.76
D-1	50.4	2.17	1.21	B-2	51.8	1.03	1.14
D-2	33.2	1.26	3.72	B-3	70.3	1.14	1.15
D-3	48.9	1.23	0.96	B-4	68.5	1.07	0.86
D-4	76.7	0.98	0.93	B-5	74.0	1.13	1.07
D-5	86.0	0.96	0.74	B-6	40.8	0.74	1.94
D-6	74.5	0.98	0.92				

[a] See formula 3 in text for numbering scheme. [b] Ppm from TMS, determined using a Varian XLFT-100 spectrometer and Digilab computer, D_2O (solvent) as internal lock, and 1,4-dioxane (δ 67.4 ppm) as internal standard. [c] Calculated as times natural abundance, by dividing intensities of individual peaks (computer calculated) in the enriched spectrum relative to average methyl carbon atom intensity in that spectrum by intensities of individual peaks in the natural abundance spectrum relative to methyl carbon atom intensity in that spectrum. Values of 1.0 ± 0.3 are regarded as within natural abundance.
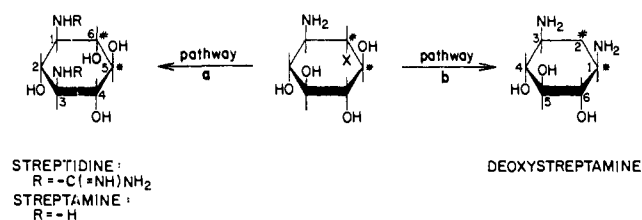

STREPTIDINE: R =-C(=NH)NH₂
STREPTAMINE: R =- H
DEOXYSTREPTAMINE

Figure 1. Proposed biosynthetic pathways from an intermediate inosamine to (a) streptamine (previous work)[19] and (b) deoxystreptamine (present report). For pathway a the intermediate inosamine has X = OH, while for pathway b X = H or OH.

some time in our laboratory.[3–6] [1-14C]Glucose, [6-14C]glucose, and [1-14C]glucosamine labeled neomycin well, but the lack of crystalline derivatives and of a completely satisfactory degradation scheme for deoxystreptamine[4,6] have led to ambiguities in the biosynthetic pathway. We have now reexamined the incorporation of glucose and glucosamine employing carbon-13 label (since the cmr spectrum itself provides evidence of sample purity and chemical degradation is unnecessary) and report a new biosynthetic pathway for deoxystreptamine.

[6-13C]Glucose (63% 13C, 2.5 g), prepared by the procedure of Schaffer and Isbell,[7] and [1-13C]glucosamine (63% 13C, 2.5 g), prepared by the procedure of Kuhn and Kirschenlohr,[8] were administered in separate experiments to cultures of *Streptomyces fradiae* (500 ml of medium in each experiment) 48 hr after inoculation. Neomycin was isolated[9] after 7 days growth (5 days after precursor addition), and neomycin B was separated[10] from small amounts of neomycin C and ne-

amine, then converted[11] to *N*-hexaacetylneomycin B (3).

Absorptions of individual carbons of 3 (Table I) were assigned[12] by off-resonance decoupling experiments, standard chemical shift data, and comparison to absorptions for the *N*-acetyl derivatives of deoxystreptamine, neamine, ribostamycin,[13] neomycin C (4), and hepta-*O*-methylneomycin B. The cmr spectra of 3 (Table I) showed that [1-13C]glucosamine labeled carbon 1 of each of the subunits (*i.e.*, B-1, C-1, D-1, R-1 in 3), while [6-13C]glucose labeled 3 at carbons B-6, C-6, D-2, and R-5. The results for the neosamines indicate both glucose and glucosamine are specific precursors, with C-1 or C-6 of the precursor becoming C-1 or C-6, respectively, of the neosamines. A more detailed description of assigned chemical shifts will appear in the microfilm edition; see paragraph at end of paper regarding supplementary material.

(1 : R = X = H ; Y = CH₂NH₂
2 : R = Y = H ; X = CH₂NH₂
3 : R = Ac ; X = H ; Y = CH₂NHAc
4 : R = Ac ; Y = H ; X = CH₂NHAc
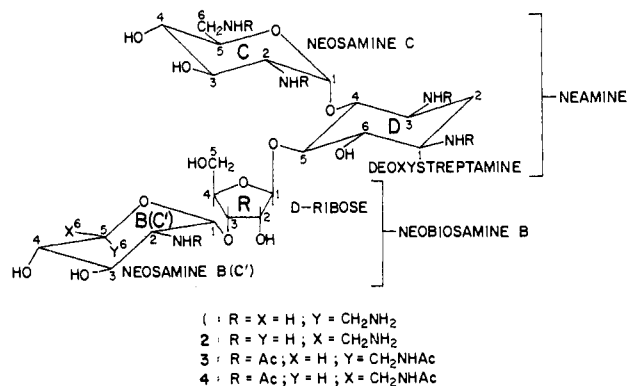
The results with the ribose moiety indicate a specific conversion of C-6 of glucose to C-5 of ribose, explicable by the hexose monophosphate pathway,[14] and suggest a conversion of C-1 of glucosamine to C-1 of ribose, which could be explained by the conversion of gluco-

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(5) R. F. Schimbor, Ph.D. Thesis, University of Illinois, Urbana, 1966.

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(12) A more detailed description of the methods used to assign chemical shifts to individual carbon atoms will be reported in the full paper.

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samine to glucose[15] and the operation in *S. fradiae* of some version of the glucuronate pathway[16] for removal of C-6 of glucose. Earlier results[3b,5] indicated that most of the label in ribose from [1-^{14}C]glucose appears at R-1.

Results for the deoxystreptamine moiety are completely unexpected. Glucosamine was proposed earlier to be converted to deoxystreptamine *via* cyclization of a 2,6-diamino-5-oxo-hexose[3,4] by a mechanism analogous to the conversion of glucose to inositol.[17] However, that mechanism would require that [1-^{13}C]glucosamine label deoxystreptamine at D-2 rather than at D-1, as is found. Cyclization of 5-oxoglucosamine and subsequent amination should also have labeled deoxystreptamine at D-2 rather than at D-1. In fact, the present results argue against any involvement of glucosamine as a direct precursor of deoxystreptamine.

The greater labeling of deoxystreptamine relative to the neosamines by glucose *vs.* the reverse pattern for glucosamine and the similar level of ^{13}C label in ribose and deoxystreptamine suggest that deoxystreptamine, like ribose, arises from glucose. A biosynthetic pathway involving D-glucose and *myo*-inosose[18] has indeed been reported for the biosynthesis of streptidine (a derivative of the diaminocyclitol streptamine, Figure 1), found in streptomycin, by *Streptomyces griseus*. However, it is carbon 5 of streptamine that has been reported[19] to be labeled by [1-^{14}C]glucose on that pathway (Figure 1, pathway a), whereas we find label from [1-^{13}C]glucosamine at deoxystreptamine D-1. Moreover, [6-^{13}C]glucose should have labeled deoxystreptamine at D-6 by pathway a, rather than at D-2. To explain our results we propose a new biosynthetic pathway (Figure 1, pathway b) involving cyclization of glucose to a cyclitol, perhaps a deoxyinosose,[20] followed by amination on the carbonyl carbon. From this stage our results would be explained if deoxystreptamine synthesis were effected by oxidation and subsequent amination at the β-carbon in the direction opposite to that reported earlier for the streptamine biosynthesis. Studies to examine the generality of pathway b are in progress.

Supplementary Material Available. A more detailed description of assigned chemical shifts 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-74-2263.

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Conformational Interactions of π Electrons

Sir:

The effect of exocyclic unsaturation on the conformational energy of substituents in six-membered rings has not been widely studied because of the difficulty in freezing out the ring reversal process in cyclohexanones.[1] Limited studies based on equilibration data have been reported.[2] A substituent in the 3 position (eq 1) should
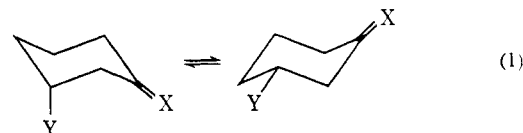

$$(1)$$

exhibit a larger preference for the axial position than in the parent cyclohexane because one of the interfering syn–axial protons has been removed. When the substituent is alkyl and when X = O, this increase in the axial conformer has been called the "3-alkylketone effect."[2] In order to study in greater detail the steric consequences of replacing a saturated CH$_2$ group by an sp^2-hybridized carbon, we have employed the *exo*-methylenecyclohexane system (eq 1, X = CH$_2$). The barrier to ring reversal in methylenecyclohexanes is about 4 kcal/mol higher than that in cyclohexanones,[1,3] so that the slow-exchange limit can easily be reached. We have found that in a polar, hydrogen-bonding solvent the proportion of axial conformer is indeed increased but that in a relatively nonpolar solvent substituents with lone pairs actually have a lower proportion of axial conformer than in the parent cyclohexane. In the latter type of solvent, the double bond can thus offer a more repulsive interaction than an axial proton. The "3-alkylketone effect" is therefore only one possible manifestation of the conformational effects of unsaturation in a six-membered ring.

In order to avoid problems associated with chemical equilibration and with bulky substituents often used to bias a ring, we have studied the equilibrium of eq 1 by the direct nmr method. The proportions of axial and equatorial conformers were obtained by integration of the resonances of the proton geminal to the substituent Y under conditions of slow ring reversal ($-120°$). The resonance of the axial methine proton (equatorial con-

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