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 $\beta$-carbon in the direction opposite to that reported earlier for the streptamine biosynthesis. Studies to examine the generality of pathway b are in progress.

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**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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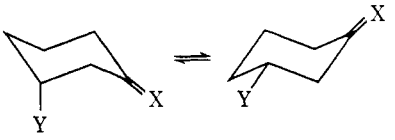
## Conformational Interactions of $\pi$ Electrons

*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-

(1) F. A. L. Anet, G. N. Chmurny, and J. Krane, *J. Amer. Chem. Soc.*, **95**, 4423 (1973).
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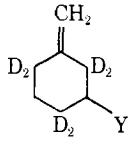
(3) J. T. Gerig and R. A. Rimerman, *J. Amer. Chem. Soc.*, **92**, 1219 (1970). The effects of endocyclic unsaturation have been studied by other authors; see F. R. Jensen and C. H. Bushweller, *ibid.*, **91**, 5774 (1969).

Table I. Substituent Preferences in 3-Substituted Methylenecyclohexanes at −120°

| Y | $K(CF_2Cl_2)$[a] | $-\Delta G°(CF_2Cl_2)$, kcal/mol | $K(CHFCl_2)$[a] | $-\Delta G°(CHFCl_2)$, kcal/mol | $A$[b] |
|---|---|---|---|---|---|
| OH | 40 ± 5[c] | 1.12 ± 0.04 | 9.9 ± 1.1 | 0.69 ± 0.03 | 0.97 |
| $OCD_3$ | 14.2 ± 0.9 | 0.80 ± 0.02 | 1.45 ± 0.05 | 0.11 ± 0.01 | 0.55 |
| OAc | 7.4 ± 0.8 | 0.61 ± 0.03 | 3.5 ± 0.2 | 0.38 ± 0.02 | 0.71 |
| OTs | | | 4.4 ± 0.7 | 0.44 ± 0.05 | |
| $SCH_3$ | 56 ± 4 | 1.22 ± 0.02 | 8.4 ± 1.0 | 0.65 ± 0.04 | 1.07 |
| $CD_3$ | 14 ± 5[d] | 0.80 ± 0.10 | 10 ± 4 | 0.70 ± 0.15 | 1.6[e] |

[a] Equatorial/axial ratio. [b] The cyclohexane $A$ value in $CS_2$ (from ref 6 and 7). [c] The quoted errors are the average deviation from the mean (3–12 runs). [d] Measured at 270 MHz (with appreciation to the Chemistry Department of the University of Chicago) by the temperature dependence of $\Delta\nu_{ae}$ for the 5-proton AB quartet over a 125° range by a computer least-squares fit; all others measured directly at 90 MHz. [e] F. A. L. Anet, C. H. Bradley, and G. W. Buchanan, *J. Amer. Chem. Soc.*, **93**, 258 (1971).

former) is invariably observed at higher field.[4] In order to remove interfering resonances and to avoid broadening from vicinal couplings, we prepared the deuterated derivative **1** for the substituents hydroxyl,

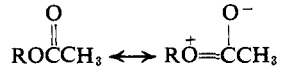


1

Y = HO, $CD_3O$, $CH_3COO$, $p$-$CH_3C_6H_4SO_2O$, $CH_3S$, $CD_3$

methoxyl, acetoxyl, tosyloxyl, methylthio, and methyl.[5] Equilibrium constants were measured in 2% solutions at −120° in a relatively nonpolar solvent ($CF_2Cl_2$) and in a more polar, hydrogen-bonding solvent ($CHFCl_2$). The results presented in Table I are compared with the equilibrium free-energy differences ($A$ values) for the same substituents in the parent cyclohexane system, as measured by Jensen and coworkers.[6] Free energies of activation for ring reversal were measured in both solvents and found to lie in the range 9.8–10.7 kcal/mol.

The expected observation of increased amounts of the axial conformer was realized for the methyl substituent. The equilibrium constant for this substituent is reduced in both solvents by an order of magnitude, from close to 100 to about 10. Thus for a nonpolar substituent, the double bond at the 3 position indeed has a smaller overall repulsive interaction than does a saturated $CH_2$ group. This property, however, is not observed in the nonpolar solvent $CF_2Cl_2$ for any of the polar or lone-pair-bearing substituents, with the qualified exception of acetoxyl. The free-energy preference for the equatorial conformation increases from the respective cyclohexane value by 0.15 kcal/mol for hydroxyl, by 0.25 for methoxyl, and by 0.15 for methylthio. We interpret these increases as resulting from a substantial repulsive interaction between the substituent with its lone-pair electrons and the double bond with its $\pi$ electrons. The magnitude of this interaction is dependent on the charge density on the atom attached to the ring. Thus in the oxygen series, similar effects are observed for hydroxyl and methoxyl. The resonance interaction in the acetoxyl group



however, draws electron density from the oxygen attached to the ring to the oxygen in the carbonyl group. As a result, the substituent–$\pi$ electron repulsion is much lower and the proportion of axial acetoxyl is actually higher in the presence of the *exo*-methylene group than in cyclohexane itself. Thus acetoxyl is "larger" in comparison to methoxyl on a cyclohexane ring, but "smaller" on a methylenecyclohexane ring in $CF_2Cl_2$. This result illustrates the relativity of the concept of substituent "sizes."

That the increased equatorial preference may result from lone-pair–$\pi$ electron repulsions rather than from dipole–dipole interactions can be seen by examination of the methylthio substituent in $CF_2Cl_2$. This relatively nonpolar group has the largest equatorial preference observed in the series. Only a very weak dipolar interaction is possible between the double bond and the C–$SCH_3$ group, but a substantial electronic repulsion must be present.

When the equilibrium constants are measured in the more polar solvent $CHFCl_2$, a considerably lower equatorial preference is observed for all the polar and lone-pair-bearing substituents. Interaction between the substituent and the solvent, in the form of direct hydrogen bonding or dipole–dipole stabilization, serves to reduce the electron density on the substituent and thereby to lower the repulsive interaction that disfavors the axial substituent. In $CHFCl_2$, all the equatorial substituent preferences are lower in the methylenecyclohexane system than in the parent cyclohexane. The expected "3-alkylketone effect" is thus found to obtain in a more polar solvent system. The general result is that substituent "sizes" for lone-pair-bearing groups on cyclohexane lie between those for methylenecyclohexanes in polar and in nonpolar solvents. Solvent effects on conformational preferences in cyclohexanes have generally been small, even for substituents such as hydroxyl.[7]

In summary, we have found that nonpolar groups such as methyl at the 3 position in methylenecyclohexane exhibit the expected increase in the proportion of the axial conformer. For polar and lone-pair-bearing

(4) The axial–proton resonance is readily identified in both deuterated and undeuterated systems by its larger band width at half-height.

(5) The full complement of deuterium was not always necessary. In some cases, deuterium could be omitted at the 6 position but in others exo-deuteration was required. All compounds gave correct elemental analyses.

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substituents in a nonpolar solvent, however, a substantial repulsive interaction with the $\pi$ electrons of the exocyclic double bond destabilizes the axial conformer, with the result that the equatorial position is favored even more for methylenecyclohexanes than for the parent cyclohexane. Lower electron densities on the substituent atom attached to the ring, as a result either of resonance delocalization to more distant atoms as in acetoxyl or of hydrogen bonding to polar solvents, result in increased amounts of the axial conformer. The "steric" interaction of $\pi$ electrons observed in the nonpolar solvent may be present in other systems and should be readily identified by its solvent dependence. Our ongoing work is involved with defining more precisely the nature of the repulsive interaction between the various substituents and the $\pi$ electrons.

(8) (a) This work was supported by the National Science Foundation (Grants GP-34259X and GP-35868X) and by the donors of the Petroleum Research Fund, administered by the American Chemical Society; (b) National Science Foundation Trainee, 1969–1970. Purchase of the 270-MHz nmr spectrometer at the University of Chicago was made possible by National Science Foundation Grant GP-33116.

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## Structure and Synthesis of Moniliformin, a Novel Cyclobutane Microbial Toxin

*Sir:*

A new microbial toxin, moniliformin, was discovered by Cole, *et al.*,[1] while screening for toxigenic products of *Fusarium moniliforme* from naturally infected southern leaf blight damaged corn seed. The original moniliformin-producing isolate of *F. moniliforme* stopped producing the toxin during the course of our study. A new source, *Gibberella fujikuroi* (perfect stage of *F. moniliforme*), was located at the American Type Culture Collection and had been assigned the ATCC #12763. The only detectable difference between strains was that the moniliformin produced by the original organism was a sodium salt, while the ATCC strain produced moniliformin containing potassium instead of sodium.

The initial chemical and spectroscopic investigations on the structure of moniliformin were inconclusive but indicated that the toxin was a small ionic molecule, possibly a carboxylate salt.[1] On treatment with trimethylsilyl chloride, no silyl derivative was formed but instead a crystalline acid, mp 158° dec, was isolated, apparently by neutralization of the salt by the HCl formed. The acid gave a positive ferric chloride test and reacted with diazomethane to form a neutral methyl derivative. Confirming the original deduction of Cole, *et al.*, the nmr spectrum of this derivative showed, in addition to a three-proton methyl singlet at $\delta$ 4.3, a one-proton singlet at $\delta$ 8.65. Consequently, moniliformin apparently possesses a single hydrogen bound to carbon.

Because the limited analytical and spectroscopic data available could not be accommodated with a reasonable structure, a single-crystal X-ray diffraction structure was carried out to provide a definitive solution.

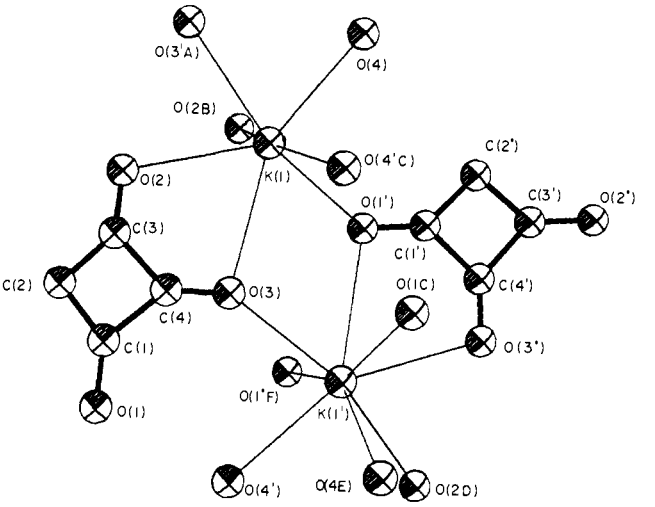


**Figure 1.** A perspective drawing of a portion of the moniliformin structure showing the environment of the 1-hydroxycyclobut-1-ene-3,4-dione anion and the potassium cations. The thin lines show all close contacts to potassium of less than 3.20 Å. Hydrogen atoms are not shown.

Crystals of moniliformin suitable for diffraction work could be grown from aqueous methanol solution. The crystals formed in the monoclinic space group $P2_1/c$, with $a = 8.46$ (1), $b = 10.918$ (9), $c = 13.28$ (1) Å, and $\beta = 100.75$ (8)°. An experimental density measurement (1.80 g/cm$^3$) suggested a fragment of molecular weight of approximately 300 as the asymmetric unit. A complete set of diffraction maxima within a $\theta$ sphere of 60° was measured with a fully automated four-circle diffractometer using Ni-filtered Cu radiation (1.5418 Å). Of the 1899 reflections measured a total of 1547 were judged observed after correction for Lorentz, polarization, and background effects. A three-dimensional Patterson synthesis was computed, and two heavy atoms, which subsequently proved to be potassium, were clearly indicated. The potassium phased electron density synthesis revealed all the remaining nonhydrogen atoms. The potassium atoms were identified by elemental analysis and the carbons and oxygens distinguished by their heights in the electron density synthesis and isotropic temperature factor refinement. Full-matrix least-squares refinements with anisotropic temperature factors for all nonhydrogen atoms lowered the conventional discrepancy index to 0.074 for the observed reflections.[2] Figure 1 is a computer-generated perspective drawing of the X-ray model.

The X-ray structural work clearly showed that crystalline moniliformin was composed of the anion of 1-hydroxycyclobut-1-ene-3,4-dione, the potassium cation, and one water of crystallization. Two crystallographically independent fragments of this composition

(1) R. J. Cole, J. W. Kirksey, H. G. Cutler, B. L. Doupnik, and J. C. Peckham, *Science*, **179**, 1324 (1973).

(2) The following library of crystallographic programs was used: C. R. Hubbard, C. O. Quicksall, and R. A. Jacobson, "The Fast Fourier Algorithm and the Programs ALFF, ALFFDP, ALFFT and FRIEDEL," USAEC Report IS-2625, Iowa State University-Institute for Atomic Research, Ames, Iowa, 1971; W. R. Busing, K. O. Martin, and H. A. Levy, "A Fortran Crystallographic Least Squares Program," USAEC Report ORNL-TM-305, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965; C. Johnson, "ORTEP, A Fortran Thermal-Ellipsoid Plot Program," U. S. Atomic Energy Commission Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965.