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Streptozocin: Structure and Chemistry[1]

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The structure of streptozocin has been shown to be that represented by **1**. Its degradation by a variety of reagents with loss of the nitroso group and formation of new ring systems is discussed, and the structures of the products are reported.

Streptozocin[2] (**1**), an antibiotic produced by *Streptomyces achromogenus* sub. *streptozoticus*, is a broad spectrum antibacterial agent.[3-4] It is also an antitumor agent being used clinically for malignant islet cell cancers of the pancreas.[5] In this report we wish to present evidence establishing the structure of streptozocin to be **1** and to discuss its chemistry.

Streptozocin (**1**) has a molecular formula of $C_8H_{15}N_3O_7$[6] established by analysis and molecular weight determination. Its ultraviolet and infrared spectra have been reported.[6] The two maxima in the ultraviolet [228 nm (ϵ 6360) and 380 nm (ϵ 136)] are consistent with the presence of an *N*-nitroso group.[7] The infrared spectrum has bands suggesting OH/NH and a carbonyl group.[6] A potentiometric titration showed the absence of titratable groups. The ^{1}H NMR spectrum of **1** was not well-resolved and could not be completely assigned, but a singlet at δ 3.15 representing 3 H indicated CH_3N. No CH_3C groups were present. A ^{13}C NMR spectrum (D_2O at pH 4.3) had chemical shifts of δ 156.7 and 28.4, confirming the presence of a carbonyl and a methyl group. The remaining signals were virtually identical with those of a mixture of α- and β-2-acetamido-2-deoxy-D-glucopyranose,[8] suggesting that D-glucosamine in the pyranose form is the nucleus of **1**.

1 is readily converted to a tetraacetyl derivative (**2**) by the acetic anhydride–pyridine procedure (Scheme I). The ^{1}H NMR spectrum of **2** showed the presence of four CH_3CO groups and the CH_3N group. A multiplet at δ 3.93–4.60 arose from 3 H on carbons substituted by oxygen or nitrogen. Two triplets (J = 10 Hz in each case) at δ 5.11 and 5.67, each representing one H, are consistent with the resonances of protons on C-3 and C-4 on a glucosamine skeleton.

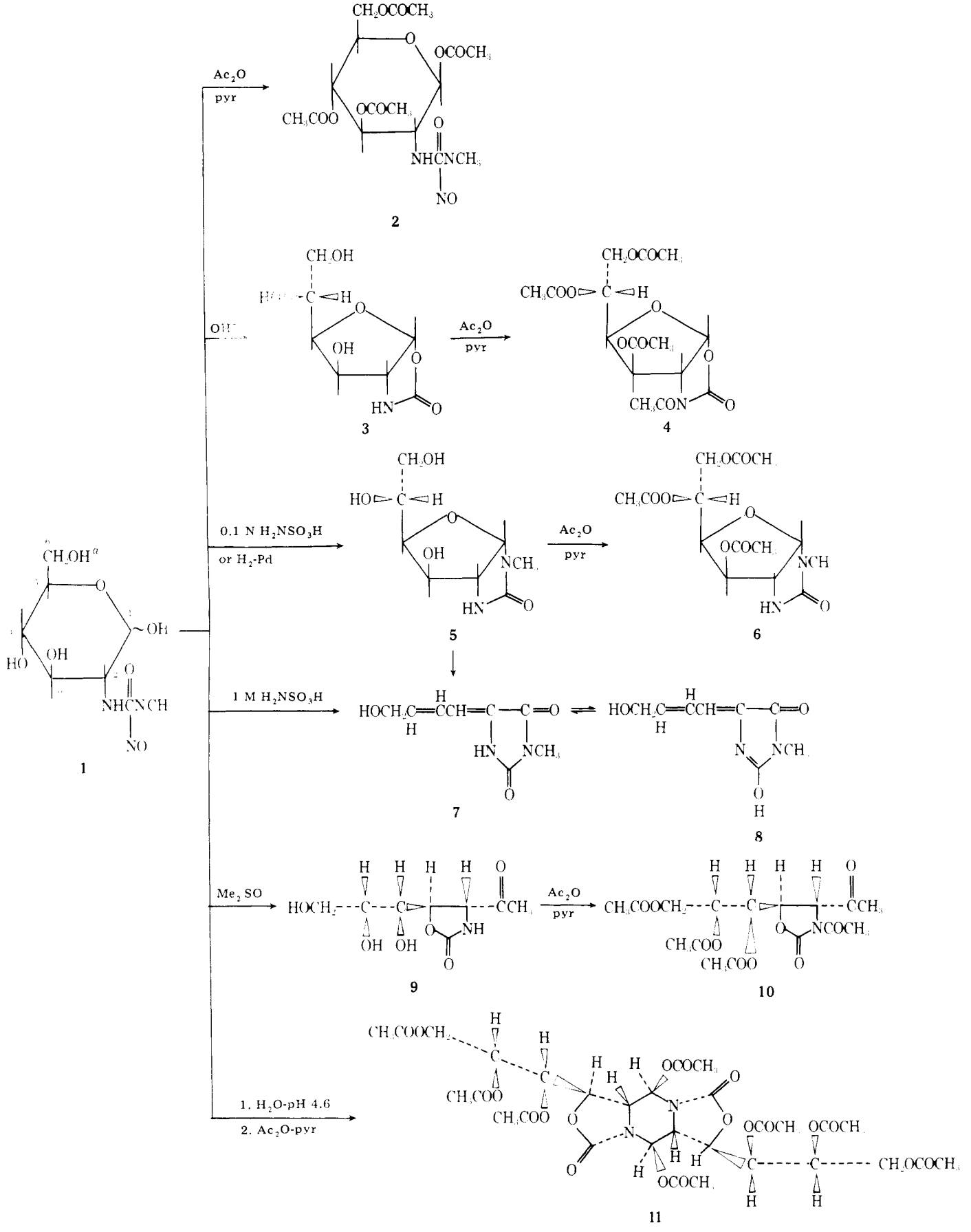
Treatment of **1** with 2 N NaOH solution gave diazomethane determined by conversion of *p*-nitrobenzoic acid to its methyl ester[6] and an amorphous solid (**3**) having the molecular formula $C_7H_{11}NO_6$. Acetylation of **3** gave a crystalline solid (**4**).[1a] Hydrolysis of both **3** and **4** with 2 N HCl gave glucosamine hydrochloride, identified by comparison with an authentic sample, and carbon dioxide. The spectral data and formation of diazomethane after treatment with alkali establish the presence in **1** of the group $CH_3N(NO)C(=O)$–. The isolation of glucosamine accounts for the remainder of the molecule. Since there is no basic group in **1**, the amine in glucosamine must have been the site of attachment of the carbonyl group in the above moiety. The conversion of **1** to a tetraacetyl derivative and its NMR spectra establish that there has been no rearrangement of the six-carbon fragment on hydrolysis. The ^{13}C NMR spectrum of **1** and mutarotation undergone by **1** from widely varying original values of different lots to a constant value of +39° in water indicate the presence of α and β forms. In fact, methyl glycosides of the two isomers have been prepared.[9] Thus, the structure of streptozocin must be as indicated in formula **1**. Acetylation gave the β isomer (**2**) as indicated by the coupling constant of 8.5 Hz for the $H_{1,2}$ coupling. The structure of **1** was confirmed by synthesis in the original work,[1a] and subsequently two improved syntheses have been reported.[10,11]

A number of degradative conversions of **1** by various reagents have been brought about, and the compound itself in various solvents such as water, ethanol, and Me_2SO undergoes spontaneous decomposition. Loss of the nitroso group occurs in all reactions of this type. Some of the degradations have already been reported,[1,12] but they will be discussed further in the present publication.

The formation of **3** from **1** as a result of base treatment has been reported,[1a] and a structure (**12**) was proposed for it with the assumption that the pyranose ring form was retained. The tetraacetyl derivative of **3** was thought to have structure **13** largely because of a 1790-cm^{-1} band in its infrared spectrum and one CH_3C signal in the ^{1}H NMR spectrum of **4** differing markedly from the other three. The infrared spectrum of **4** has three bands in the carbonyl region at 1790, 1735, and 1705 cm^{-1}, suggesting acetate (1735 cm^{-1}), a carbonyl similar to the one in **3** (1705 cm^{-1}), and a new group giving the high wavenumber band. The ^{1}H NMR spectrum has signals at δ 1.95, 2.01, and 2.03 which must be from acetyl CH_3C groups attached to oxygen, while a fourth resonance at δ 2.42 suggests some other type of acetyl group. The other chemical shifts were as expected for a sugar derivative except that the $H_{2,3}$ coupling was zero, perhaps indicating a furanose ring. The ^{13}C NMR spectrum was reasonable for both **4** and **13**. As a result of inconsistencies in the data derived from the alkaline degradation product of **1** and its acetyl derivative, an X-ray crystallographic study was done on the acetyl derivative. Its structure was established as that represented by **4**, and, as acetylation should not cause rearrangement of the ring structure, the structure of the initial degradation product

0022-3263/79/1944-0009$01.00/0

Scheme I



[a] Numbering in all compounds is the same as that of the corresponding atoms in 1.[2]

Table I. Final Atomic Parameters ($\times 10^4$) and Standard Deviations (in Parentheses)[a]

	X	Y	Z	B11	B22	B33	B12	B13	B23
O(1)	-4 (2)	4207 (1)	1568 (1)	80 (2)	54 (1)	15 (1)	14 (3)	12 (2)	3 (1)
C(2)	873 (3)	3668 (2)	959 (1)	55 (3)	54 (2)	15 (1)	-3 (4)	4 (3)	-7 (2)
C(3)	1083 (3)	2276 (2)	1200 (1)	47 (3)	55 (2)	16 (1)	-16 (4)	2 (3)	-5 (2)
C(4)	914 (3)	2323 (2)	2131 (1)	47 (3)	54 (2)	17 (1)	-6 (4)	-1 (3)	1 (2)
N(5)	2203 (3)	2331 (2)	2552 (1)	65 (3)	62 (2)	16 (1)	-11 (4)	-5 (3)	-3 (2)
C(6)	2518 (3)	3535 (3)	2846 (1)	100 (4)	77 (2)	16 (1)	-46 (6)	-2 (4)	-4 (2)
O(6)	3530 (2)	3885 (2)	3191 (1)	126 (3)	104 (2)	28 (1)	-59 (5)	-47 (3)	-15 (2)
O(7)	1470 (2)	4338 (2)	2674 (1)	117 (3)	56 (2)	19 (1)	-12 (4)	-16 (3)	-10 (2)
C(8)	345 (3)	3649 (2)	2304 (1)	81 (3)	58 (2)	15 (1)	-3 (5)	2 (3)	3 (2)
C(9)	175 (3)	3897 (2)	140 (1)	58 (3)	60 (2)	16 (1)	-1 (4)	9 (3)	-2 (2)
C(10)	-178 (3)	5285 (2)	1 (2)	67 (3)	65 (2)	20 (1)	10 (5)	-4 (4)	-3 (2)
O(11)	998 (2)	6063 (2)	187 (1)	76 (2)	51 (1)	21 (1)	11 (3)	14 (2)	-6 (2)
C(12)	847 (3)	6999 (2)	739 (2)	84 (3)	46 (2)	19 (1)	26 (4)	9 (4)	3 (2)
O(12)	-234 (2)	7288 (2)	1032 (1)	90 (2)	77 (2)	27 (1)	33 (4)	31 (3)	-18 (2)
C(12M)	2169 (4)	7591 (3)	931 (2)	92 (4)	61 (2)	38 (1)	0 (5)	11 (4)	-20 (3)
O(13)	1172 (2)	3472 (2)	-446 (1)	53 (2)	59 (1)	15 (1)	4 (3)	4 (2)	-7 (2)
C(14)	706 (3)	2836 (2)	-1103 (1)	72 (3)	55 (2)	15 (1)	10 (4)	-8 (3)	-2 (2)
O(14)	-473 (2)	2580 (2)	-1203 (1)	58 (2)	108 (2)	23 (1)	-6 (4)	-18 (2)	-29 (2)
C(14M)	1846 (3)	2507 (3)	-1655 (2)	76 (3)	78 (3)	21 (1)	25 (5)	-2 (4)	-13 (3)
O(15)	5 (2)	1495 (1)	888 (1)	52 (2)	51 (1)	21 (1)	-8 (3)	-1 (2)	-14 (2)
C(16)	343 (3)	612 (2)	320 (1)	64 (3)	47 (2)	20 (1)	12 (4)	-6 (3)	-7 (2)
O(16)	1484 (2)	466 (2)	73 (1)	63 (2)	75 (2)	29 (1)	13 (3)	6 (3)	-33 (2)
C(16M)	-873 (4)	-94 (3)	57 (2)	70 (3)	63 (2)	28 (1)	-12 (4)	-7 (4)	-14 (3)
C(17)	2940 (3)	1194 (3)	2613 (1)	71 (3)	74 (2)	19 (1)	4 (5)	14 (4)	20 (2)
O(17)	2508 (2)	272 (2)	2255 (1)	81 (2)	56 (2)	31 (1)	6 (3)	-2 (3)	12 (2)
C(17M)	4193 (3)	1179 (4)	3129 (2)	74 (4)	121 (3)	29 (1)	10 (6)	-22 (4)	26 (3)

[a] The form of the anisotropic temperature factors is $\exp[-B_{11}h^2 - B_{22}k^2 - B_{33}l^2 - B_{12}hk - B_{13}hl - B_{23}kl]$.

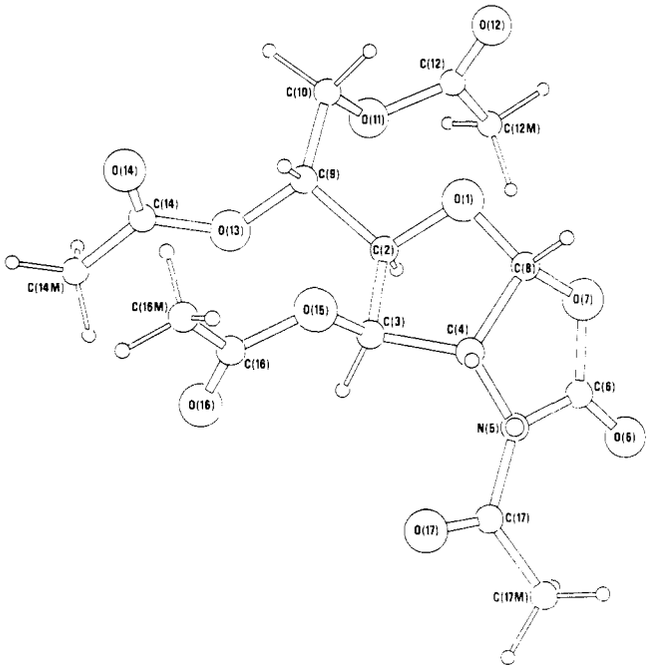
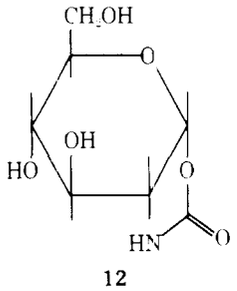
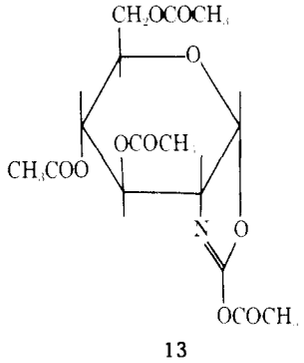


Figure 1. Numbering, conformation, and configuration of **4**. The configuration is 2*S*,3*R*,4*R*,8*R*,9*R*.



12



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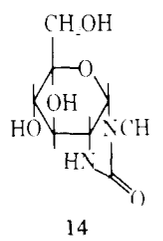
must be as in **3**.

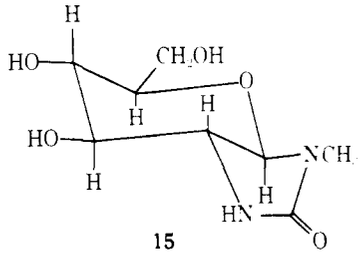
Details of the crystallographic analysis of **4** are in the Experimental Section. Figure 1 shows numbering, conformation, and configuration at the five carbon centers. The enantiomorph assignment was made on the basis of the known absolute configuration of glucosamine, which is the acid degradation product of **4**. Final atomic coordinates and thermal parameters for nonhydrogen atoms are given in Table I, and bond lengths and angles are listed in Tables II and III. Table IV gives hydrogen coordinates.[13]

The furan ring is in a twist conformation rather than an envelope. In the view shown in Figure 1, O(1) is up and C(2) is down from the plane on the other three ring atoms. The oxazolidine ring and the acetyl on N(5) are nearly planar; torsion angles range from 3 to 10°.

The 0.1 M sulfamic acid degradation product (**5**) of **1** was reported in an earlier publication,[1] and the structure **14** was originally proposed. This structure has the α configuration, but there was no specific discussion of chirality at C-1. In a subsequent publication[12] an isomer of **5** was reported whose spectral data and rotation suggested for it the structure **14**. In view of this it was hypothesized that **5** was the α isomer **15**.



14



15

The realization that the base conversion product (**3**) contained a furanose ring caused us to reexamine the structure of the acid-derived product. In addition to the sulfamic acid procedure, **5** is obtained by catalytic reduction of **1**, although chemical reduction gives a different product (**16**).[12] Cyclization of the intermediate urea prepared in the synthesis[10] of **1** also gives **5**.[12]

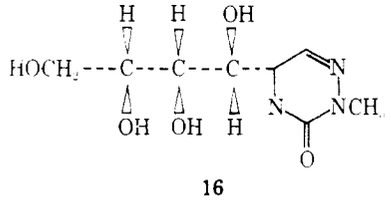


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Table II. Bond Lengths (Å) and Standard Deviations (in Parentheses)

O(1)	C(2)	1.438(3)
O(1)	C(8)	1.391(3)
C(2)	C(3)	1.529(3)
C(2)	C(9)	1.533(4)
C(3)	C(4)	1.546(3)
C(3)	O(15)	1.434(3)
C(4)	N(5)	1.444(4)
C(4)	C(8)	1.527(4)
N(5)	C(6)	1.389(4)
N(5)	C(17)	1.399(4)
C(6)	O(6)	1.201(4)
C(6)	O(7)	1.360(4)
O(7)	C(8)	1.454(4)
C(9)	C(10)	1.515(4)
C(9)	O(13)	1.446(3)
C(10)	O(11)	1.447(4)
O(11)	C(12)	1.348(3)
C(12)	O(12)	1.204(4)
C(12)	C(12M)	1.473(5)
O(13)	C(14)	1.354(3)
C(14)	O(14)	1.199(3)
C(14)	C(14M)	1.483(4)
O(15)	C(16)	1.359(3)
C(16)	O(16)	1.202(3)
C(16)	C(16M)	1.470(4)
C(17)	O(17)	1.210(3)
C(17)	C(17M)	1.495(5)

Table III. Bond Angles (Degrees) and Standard Deviations (in Parentheses)

C(2)	O(1)	C(8)	107.2(2)
O(1)	C(2)	C(3)	105.9(2)
O(1)	C(2)	C(9)	106.6(2)
C(3)	C(2)	C(9)	116.0(2)
C(2)	C(3)	C(4)	102.3(2)
C(2)	C(3)	O(15)	110.7(2)
C(4)	C(3)	O(15)	107.1(2)
C(3)	C(4)	N(5)	112.6(2)
C(3)	C(4)	C(8)	104.7(2)
N(5)	C(4)	C(8)	103.0(2)
C(4)	N(5)	C(6)	111.6(2)
C(4)	N(5)	C(17)	118.8(2)
C(6)	N(5)	C(17)	129.5(3)
N(5)	C(6)	O(6)	128.8(3)
N(5)	C(6)	O(7)	108.8(2)
O(6)	C(6)	O(7)	122.3(3)
C(6)	O(7)	C(8)	110.7(2)
O(1)	C(8)	C(4)	108.1(2)
O(1)	C(8)	O(7)	110.1(2)
C(4)	C(8)	O(7)	104.7(2)
C(2)	C(9)	C(10)	112.7(2)
C(2)	C(9)	O(13)	103.8(2)
C(10)	C(9)	O(13)	110.5(2)
C(9)	C(10)	O(11)	109.1(2)
C(10)	O(11)	C(12)	117.8(2)
O(11)	C(12)	O(12)	123.4(3)
O(11)	C(12)	C(12M)	110.8(3)
O(12)	C(12)	C(12M)	125.7(2)
C(9)	O(13)	C(14)	117.3(2)
O(13)	C(14)	O(14)	123.1(2)
O(13)	C(14)	C(14M)	110.5(2)
O(14)	C(14)	C(14M)	126.3(2)
C(3)	O(15)	C(16)	117.2(2)
O(15)	C(16)	O(16)	123.2(2)
O(15)	C(16)	C(16M)	110.4(2)
O(16)	C(16)	C(16M)	126.3(2)
N(5)	C(17)	O(17)	117.7(3)
N(5)	C(17)	C(17M)	118.3(3)
O(17)	C(17)	C(17M)	123.8(3)

Analysis and molecular weight determinations established a molecular formula of $C_8H_{14}N_2O_5$ for **5**, and thus it is formed from the urea with the loss of the elements of one molecule of water, which must occur by cyclization to C-1 or C-3 of the urea. The infrared spectrum showed the presence of a carbonyl group (1650 cm^{-1} band) as well as OH/NH. The ^{13}C NMR spectrum also indicated a carbonyl (δ 163.5), an anomeric carbon (δ 92.8), and the carbons C-2 through C-6 of **1** as well as the CH_3N carbon. The ^1H NMR spectrum of **5** was very reminiscent of that of **4** after considering differences due to acetylation. Coupling constants were very similar, including the $H_{2,3}$ coupling constant of zero.

In order to get a better comparison, **5** was acetylated using acetic anhydride–pyridine to give a crystalline compound **6** containing only three acetyl groups with all attached to oxygen as shown by NMR and mass spectra. The resonance of the anomeric proton in the ^1H NMR spectrum of **6** was very close to that of the same proton in the spectrum of **5** (δ 5.55 and 5.69, respectively), establishing that acetylation had not occurred at the anomeric carbon. Consequently, there must be no hydroxyl on this carbon, showing that cyclization had occurred to C-1 of **1** giving a carbon–nitrogen bond as in structure **5**. A comparison of the ^1H NMR spectra of **4** and **6** showed a striking similarity. The chemical shifts of the various protons were only moderately close, but coupling constants were almost identical except for $J_{1,2}$ (Table V), establishing that **6** also has the two fused five-membered rings. Furthermore, in the ^1H NMR spectrum of the unacetylated compound **5**, a signal at δ 3.48 arises from the proton on C-5, while in the spectrum of **6** the same proton gives a peak at δ 5.22. Thus, acetylation of a hydroxyl group on C-5 must have occurred, certainly indicating a furanose ring in **6** and very probably in **5**. The resonance at δ 4.17 in the ^1H NMR spectrum of **6** arising from hydrogen on C-4 suggests that no acylation occurred at this carbon, which could only be due to its oxygen atom being part of the furanose ring. These results establish that the acetyl derivative of the acid degradation product of **1** has the structure shown as **6**.

Table IV. Final Atomic Coordinates for Hydrogen Atoms ($\times 10^3$) and Standard Deviations (in Parentheses)

	X	Y	Z
H(2)	173 (3)	411 (3)	96 (2)
H(3)	201 (3)	193 (3)	102 (2)
H(4)	32 (3)	167 (3)	233 (2)
H(8)	-46 (3)	368 (3)	270 (2)
H(9)	-64 (3)	335 (3)	11 (2)
H(10A)	-101 (3)	551 (3)	35 (2)
H(10B)	-49 (3)	549 (3)	-61 (2)
H(12A)	209 (4)	843 (3)	119 (2)
H(12B)	268 (4)	774 (3)	49 (2)
H(12C)	275 (4)	711 (3)	128 (2)
H(14A)	152 (3)	204 (3)	-212 (2)
H(14B)	251 (3)	203 (3)	-140 (2)
H(14C)	223 (3)	332 (3)	-185 (2)
H(16A)	-64 (3)	-86 (3)	-25 (2)
H(16B)	-147 (3)	39 (3)	-30 (2)
H(16C)	-144 (4)	-23 (3)	37 (2)
H(17A)	475 (4)	35 (3)	298 (2)
H(17B)	384 (4)	122 (3)	365 (2)
H(17C)	475 (4)	195 (3)	307 (2)

Table V. Coupling Constants of 4 and 6

	4	6
$J_{1,2}$	6.5	5.3
$J_{2,3}$	0	0
$J_{3,4}$	3.0	3.1
$J_{4,5}$	9.0	9.1
$J_{5,6\alpha}$	2.3	3.1
$J_{5,6\beta}$	5.5	6.0
$J_{6\alpha,6\beta}$	12.0	12.5

Stronger sulfamic acid (1 M) causes much more extensive changes in 1. The product is an orange solid (7) which also arises from a similar acid treatment of 5.[1b] Analytical data and mass spectra established a molecular formula of $C_8H_{10}N_2O_3$ for 7. The ultraviolet spectrum of 7 (λ_{max} 310 nm) and its color suggest an extended system of conjugated double bonds. The infrared spectrum has bands at 1765, 1700, and 1655 cm^{-1} in the carbonyl region, typical of a hydantoin moiety with a group attached by a double bond.[14] The infrared spectrum also has bands at 3440 and 3200 cm^{-1}, typical of OH and NH stretching, respectively. The ^1H NMR spectrum shows the presence of ten hydrogen atoms of which three are part of a CH_3N group (δ 3.08) and two are exchangeable. A broad signal at δ 10.20 suggests that an enolic H is present, as in 8, in solution but not in the crystal form on the basis of the infrared spectrum. Spin decoupling ^1H NMR experiments establish that resonances at δ 7.18, 6.54, and 6.38, each due to one H, are on a three-carbon system all of which are olefinic and that two of these H are adjacent and trans to each other. One exchangeable H is coupled with a methylene group which is in turn coupled with one of the trans olefinic protons. Thus, the group *trans*-$HOCH_2CH{=}CHCH{=}$ is present. Combination of this moiety with the hydantoin system and considering the structure of the precursor (5) of 7 suggest the proposed structure represented as 7. The ^{13}C NMR spectra, including off-resonance decoupled experiments, are in complete agreement with such a structure as 7. Eight carbon resonances were present, one of which was CH_3N (δ 24.1), one of which carried two hydrogens and was substituted by O or N (δ 61.2), three of which were attached to one hydrogen, and three to none. Two of the latter resonating at δ 163.7 and 154.3 were assigned to the carbonyl groups, and the other four with one or no H had chemical shifts indicating they were olefinic carbons atoms.

Alkaline degradation of 7 resulted in isolation of products accounting for one carbonyl as carbon dioxide, the CH_3N as methyl amine, two carbons as glyoxal, and two carbons as acetaldehyde. The latter two were isolated as 2,4-dinitrophenylhydrazones. Such products could result from hydration of the conjugated double bond system of 7 followed by retroaldol reactions. In addition, glycine should be formed, but it was not isolated. It, then, is clear that only structures 7 and 8 are consistent with the above findings. It is probable that 1 is first converted to 5 as an intermediate. Protonation of a hydroxyl group is then followed by ring opening and dehydration.

In Me_2SO, 1 undergoes a rapid exothermic decomposition with evolution of a gas, presumably N_2 since the product 9 contains only one nitrogen. The material isolated was an amorphous solid which was never obtained completely pure, and analytical results were poor. Its infrared spectrum showed a broad OH/NH band at 3360–3250 cm^{-1} and a broad carbonyl band centered at 1745 cm^{-1}. The ^{13}C NMR spectrum had resonances indicating eight carbon atoms, one of which was a ketone carbonyl (δ 209.6). A second carbonyl was present, but the anomeric carbon had disappeared. A signal at δ 28.0 most probably arose from a CH_3C group as the ^1H NMR had a singlet at δ 2.38 due to 3 H, suggesting $CH_3C{=}O$. The remaining five peaks were assigned to carbons substituted by O or N. A mass spectrum did not conclusively establish the molecular formula, and analyses were ambiguous; but the molecular formula of $C_8H_{13}NO_6$ seemed probable. Conversion to an acetyl derivative gave a crystalline material (10) having a molecular formula of $C_{16}H_{21}NO_{10}$ established by analysis and a mass spectrum. The ^1H NMR spectra showed the presence of four acetyl groups. The ^{13}C NMR spectrum again indicated the presence of a ketone carbonyl which was confirmed by formation of a 2,4-dinitrophenylhydrazone derivative. The expected signals arising from four acetyl methyl carbons and four acetyl carbonyl carbons were present. The remaining signals were much as were those in the ^{13}C NMR spectrum of 9. The infrared spectrum of 10 showed no band for OH/NH, but four carbonyl bands were present at 1785, 1755, 1730, and 1695 cm^{-1}. The 1785-cm^{-1} band is similar to one of the carbonyl bands in 4 and indicates the presence of acetyl on nitrogen already acyl substituted. The ^1H NMR spectrum confirms the presence of four acetyl groups and an additional methyl group attached to an sp^2 carbon. The ^{13}C NMR signals at δ 160.0 for 9 and δ 151.7 for 10 are so close to that of the carbamate carbonyl in 4 that it very strongly points to the presence of such a carbonyl in 9 and 10. The 1785-cm^{-1} infrared carbonyl band would also suggest a grouping such as the $CH_3CONCOO$ found in 4. The remaining three acetyl groups must be on oxygen and, thus, there could have been only three hydroxyl groups in 9. The molecular formula of 9 as deduced from 10 requires the presence of a ring in addition to the carbonyl groups. The accumulated evidence of these data points conclusively to structures 9 and 10 for the structures of the product derived from Me_2SO decomposition of 1 and for the acetyl derivative of that product.

Although 1 is most stable at pH 4,[6] it decomposes in saline solution at pH 4.6 to give a mixture of products which are resistant to separation. Acetylation of the mixture gave a material which was separated chromatographically into three components, one of which is still a mixture of two compounds. Purification of the first material off the column gave a crystalline solid (11) which was characterized and identified, but the remaining products were not purified sufficiently to establish their structure. Analysis of the purified material (11) suggested an empirical formula of $C_{15}H_{19}NO_{10}$. The NMR spectra were consistent with such a molecular formula for 11, but the mass spectra established that such a formula could not be correct. A high resolution mass spectrum of 11 gave *m/e* 686.1853 for the largest ion, which would represent a composition of $C_{28}H_{34}N_2O_{18}$. The previously discussed data rule this out, and the only reasonable hypothesis was that 11 was a dimer of $C_{15}H_{19}NO_{10}$ which gave $M^+ - 60$ as the highest ion as a result of the loss of one molecule of acetic acid. If this were the case, the NMR spectra would require 15 pairs of identical carbon atoms and 19 pairs of identical hydrogen atoms. Such requirements could be met by the structure depicted in 11. The anomeric carbon atoms were assigned the ^{13}C NMR resonance at δ 79.3, which is slightly upfield for a normal anomeric carbon atom but is very close to that of the anomeric carbon atom in nojirimycin (α anomer δ 82.6, β anomer δ 79.0),[15] which is a compound having the N–C–O system believed to be present in 11. The acetoxy groups on the six-membered ring must be cis to each other, as any trans arrangement would necessitate either hydrogen atoms on these carbon atoms differing in relation to adjacent hydrogens or the carbon atoms would not be identical. In fact, any chair conformation would be subject to such limitations. Thus, it seems probable that this acetate has the structure 11, in which the six-membered ring has the boat conformation, *cis*-acetoxy groups, and because of the anomeric proton coupling constant, at least quasiequatorial acetoxy groups.

Experimental Section

^{13}C NMR spectrum of 1 (D_2O at pH 4.3): 157.6, 156.7 (C=O), 96.1, 92.3 (C-1), 59.4, 56.7 (C-2), 75.0, 72.2 (C-3), 71.5, 71.4 (C-4, 77.3, 72.9 (C-5), 62.1, 62.0 (C-6), 28.4 (CH_3N), β and α series, respectively.

Tetra-*O*-acetylstreptozocin (2). A mixture of 5 g of 1 and 100 mL of $(CH_3CO)_2O$-pyr (1:1) was warmed until solution occurred. After 18 h at room temperature, the solution was added to 500 mL of ice-cold H_2O. The precipitate which formed was removed and dissolved in ethyl acetate. This solution was washed with two 50-mL portions of cold H_2O, 50 mL of cold 0.5 N HCl, 50 mL of cold H_2O, 50 mL of cold saturated $NaHCO_3$, and 50 mL of H_2O. The dried ($MgSO_4$) solution was evaporated to dryness in vacuo to give 4.9 g of crystalline

solid: mp 114–118 °C; R_f 0.51 (SiO_2; $CH_3COOC_2H_5$–H_2O, 98:2); $[\alpha]_D$ + 50° (*c* 1, $CHCl_3$); IR (Nujol) 3280, 1745, 1715, 1540, 1255, 1220, 1085, 1045, 990, 910, and 795 cm^{-1}; ^1H NMR ($CDCl_3$) 1.99, 2.05, 2.08 (3 s, 12 H, 4 CH_3CO), 3.13 (s, 3 H, CH_3N), 3.75–4.65 (m, 4 H, CHO, CHN), 5.17 (t, 1 H, *J* = 9.0 Hz, H-4), 5.53 (t, 1 H, *J* = 9.0 Hz, H-3), 5.97 (d, 1 H, *J* = 8.5 Hz, anomeric H), 7.56 (d, 1 H, *J* = 9.8 Hz, NH).

Anal. Calcd for $C_{16}H_{23}N_3O_{11}$: C, 44.19; H, 5.31; N, 9.69; O, 40.51. Found: C, 44.29; H, 5.11; N, 9.41; O, 39.91.

[3a*R*-(3aα,5α,6α,6aα)]-5-(1,2-dihydroxyethyl)-3a,5,6,6a-tetrahydro-6-hydroxyfuro[3,2-*d*]oxazol-2(1*H*)-one (3). A solution of 8 g of **1** in 320 mL of 1 N NaOH was allowed to stand at room temperature for 16 h. The solution was adjusted to pH 7.0 with 6 N HCl and evaporated to dryness in vacuo. The residue was dissolved in 125 mL of H_2O and put on a column consisting of a mixture of 120 g of activated carbon and 240 g of diatomaceous earth, and the column was washed with 3 L of H_2O. It was eluted with 2 L of H_2O–CH_3COCH_3 (99:1) followed by 5 L of H_2O–CH_3COCH_3 (95:5). The fractions containing product were combined as determined by weight analysis and ninhydrin reagent. They were in the 95:5 eluate. Solid was isolated by evaporation of the pooled fractions in vacuo followed by lyophilization: yield of amorphous solid 2.37 g; mp 165–168 °C dec; $[\alpha]^{25}_D$ 40.7° (*c* 1, H_2O); IR (Nujol) 1725 cm^{-1}.

Anal. Calcd for $C_7H_{11}NO_6$: C, 41.01; H, 5.41; N, 6.83; O, 46.83. Found: C, 41.28; H, 6.06; N, 6.88; O, 46.13.

Acid Hydrolysis of 3. A solution of 500 mg (2.6 mmol) of **3** in 30 mL of 1 N HCl was boiled for 1 h. During the hydrolysis N_2 was passed through the reaction mixture and into $Ba(OH)_2$ solution. The precipitated $BaCO_3$ was isolated by filtration, washed, and dried: yield 435 mg, 0.85 mol per mole of **3**. The cooled hydrolysate was decolorized with activated charcoal and then mixed with 800 mL of acetone. The colorless needles which precipitated were removed by filtration: yield 135 mg (21.5%). Their rotation and infrared and ^1H NMR spectra were identical with those of glucosamine hydrochloride.

[3a*R*-(3aα,5α,6α,6aα)]-5-(1,2-diacetyloxyethyl)-3a,5,6,6a-tetrahydro-6-(acetyloxy)furo[3,2-*d*]-1-acetyloxazol-2(1*H*)-one (4). After a solution of 2.8 g of **3** in 60 mL of $(CH_3CO)_2O$-pyr (1:1) had stood for 20 h at room temperature, it was concentrated under reduced pressure to a volume of 10 mL. The residue was mixed with 100 mL of H_2O and extracted with three 50-mL portions of ethyl acetate. The combined extracts were washed with 50 mL of H_2O, 50 mL of 0.5 N HCl, 50 mL of 0.5 N $NaHCO_3$, and 50 mL of H_2O. The organic solution was dried (Na_2SO_4) and concentrated in vacuo to dryness to give 3.6 g of residue. The product was purified by partition chromatography using diatomaceous earth and the solvent system cyclohexane–ethyl acetate–ethanol–McIlvaine's pH 5.0 buffer (3:1:2:2). The above residue dissolved in 10 mL of lower phase was mixed with 20 g of diatomaceous earth and added to the top of a column of 200 g of diatomaceous earth mixed with 80 mL of lower phase and 1100 mL of upper phase. The column was eluted with upper phase, collecting 20-mL fractions and analyzing by weight. On this basis, fractions 35–70 were combined and evaporated to dryness in vacuo to give 2.20 g of amorphous material. The residue was triturated in 10 mL of EtOH, and the resulting crystalline material was recrystallized from EtOH: yield 920 mg; mp 169–172 °C; $[\alpha]^{25}_D$ −46° (*c* 1, EtOH); IR (Nujol) 1790, 1745, 1705, 1330, 1300, 1240, 1225, 1210, 1170, 1125, 1110, 1065, 1045, 1035, 1000, 985, 935, 895, 875, 785, and 758 cm^{-1}; ^1H NMR (Me_2SO-d_6) 1.97, 2.03, 2.07 (3, s, 9 H, $3CH_3COO$), 2.48 (s, 3 H, CH_3CON), 4.05 (d of d, 1 H, *J* = 6.0, 12.5 Hz, H-6α), 4.43 (d of d, 1 H, *J* = 3.1, 12.5 Hz, H-6β), 4.51 (d of d, 1 H, *J* = 3.1, 9.1 Hz, H-4), 4.63 (d, 1 H, *J* = 5.3, 0 Hz, H-2), 5.09 (m, 1 H, *J* = 9.1, 3.1, 6.0 Hz, H-5), 5.6 (d, 1 H, *J* = 0, 3.1 Hz, H-3), 6.3 (d, 1 H, *J* = 5.3 Hz, anomeric H); ^{13}C NMR (Me_2SO-d_6) 170.6, 169.5, 169.2, 168.2 ($4CH_3CO$), 152.0 [NC(O)O], 99.7 (anomeric), 77.4 (C-4), 71.9 (C-5), 66.6 (C-3), 64.1 (C-2), 62.5 (C-6), 23.5 (CH_3CON), 20.4 (3 CH_3COO), mass spectrum *m/e* 329.1091 ($M^+ - CO_2$) (calcd for $C_{14}H_{19}NO_8$, 329.1111).

Anal. Calcd for $C_{15}H_{19}NO_{10}$: C, 48.25; H, 5.09; N, 3.75; O, 42.89. Found: C, 48.36; H, 5.31; N, 3.73; O, 42.76.

Acid Hydrolysis of 4. This was done in a manner very similar to the acid hydrolysis of **3** but using 270 mg (0.72 mmol). The yield of $BaCO_3$ was 128 mg (0.65 mmol, 90%). The crystalline product from CH_3COCH_3–H_2O was recrystallized from H_2O–CH_3COCH_3–CH_3OH to give 116 mg (75%) of glucosamine hydrochloride identified by rotation and infrared and ^1H NMR spectra.

Anal. Calcd for $C_6H_{13}NO_5$·HCl: C, 33.52; H, 6.56; N, 6.52; Cl, 16.49. Found: C, 33.91; H, 6.62; N, 6.17; Cl, 16.40.

[3a*S*-(3aα,5α,6α,6aα)]-5-(1,2-dihydroxyethyl)-1,3,3a,4,5,6a-hexahydro-6-hydroxy-3-methyl-2*H*-furo[2,3-*d*]imidazol-2-one (5). A solution of 5.0 g of **1** in 250 mL of 0.1 M H_2NSO_3H was allowed to stand at room temperature in the dark for 24 h. It was then passed over an excess of Dowex-1,X-2 (HCO_3^-) ion-exchange resin. The effluent was evaporated to dryness in vacuo. The residue was crystallized from 5 mL of CH_3OH and recrystallized from 95% EtOH: yield 800 mg; mp 177–178 °C; $[\alpha]_D$ −21° (*c* 0.768, H_2O); IR (Nujol) 3270, 3095, 2950, 2900, 1650, 1500, 1345, 1295, 1265, 1250, 1155, 1130, 1090, 1030, 975, 885, 795, and 765 cm^{-1}; ^1H HMR (D_2O) 2.66 (s, 3 H, CH_3N), 3.47 (m, 1 H, *J* = 8.8, 2.2, 6.0 Hz, H-5), 3.67 (d of d, 1 H, *J* = 6.0, 12.2 Hz, H-6β), 3.79 (d of d, 1 H, *J* = 2.5, 8.8 Hz, H-4), 3.83 (d of d, 1 H, *J* = 2.2, 12.2 Hz, H-6α), 4.18 (d, 1 H, *J* = 6.0 Hz, H-2), 4.26 (d, 1 H, *J* = 0, 2.5 Hz, H-3), 5.69 (d, 1 H, *J* = 6.0 Hz, anomeric H); ^{13}C NMR (D_2O) 163.5 (C=O), 92.8 (anomeric), 79.7, 76.1 (C-4 and C-5), 69.9 (C-3), 64.9 (C-2), 62.4 (C-6), 28.4 (CH_3N).

Anal. Calcd for $C_8H_{14}N_2O_5$: C, 44.07; H, 6.47; N, 12.85; O, 36.70; mol wt, 218. Found: C, 44.23; H, 6.69; N, 12.86; O, 36.76; mol wt 197 (isothermal distillation).

5 consumes 1 mol of IO_4^-/mol with the titration carried out as previously reported.[16]

X-ray Crystallography of 4. Crystals of **4**, $C_{15}H_{19}NO_{10}$, were large clear orthorhombic prisms, space group $P2_12_12_1$, as evidenced by absence of $h00$, $0k0$, and $00l$ reflections for h, k, or l odd. Crystal data are a = 9.819 (2), b = 10.501 (2), c = 16.503 (2) Å, Z = 4, D_m = 1.41 g cm^{-3}, D_c = 1.45 g cm^{-3}, μ(Cu Kα) = 9.7 cm^{-1}.

Intensity data on the 1408 reflections with $2\theta \leq 138°$ were measured at low temperature (about −155 °C) using step scans[17] at 1°/min with scan ranges ≥ 3.4° in 2θ on a Syntex $P\bar{1}$ diffractometer controlled by an IBM 1800 computer. Graphite monochromatized Cu Kα radiation was used (λ 1.5418 Å). Accurate cell parameters were determined automatically using a least-squares calculation based on very accurately determined Kα_1 2θ values for 20 selected high 2θ angle reflections.[17] Standard deviations in observed intensities were approximated by the function $\sigma^2(I) = \sigma^2_{\text{counting statistics}} + (0.013I)^2$, where the coefficient of I was calculated from intensities of ten reflections monitored throughout the data collection, considering deviations in intensities which were not explained by counting statistics. The usual Lorentz correction was made along with a polarization correction appropriate for a monochromator with 50% perfect character.[18]

The structure was solved by direct methods using a program written by David J. Duchamp of The Upjohn Company. A new version of the program, DIREC II, which uses quartets,[19] was used. Coordinates (including hydrogen coordinates), anisotropic thermal parameters for heavier atoms, a scale factor, and a secondary extinction parameter were refined by crystallographic least squares minimizing the function $-\Sigma w(F_o^2 - F_c^{*2})^2$ where weights w were taken as the reciprocals of the variances $\sigma^2(F_o^2)$ and where F_c^* was as defined by Larson.[20] Anomolous components were included for C, N, and O atoms using the parameters of Cromer and Liberman.[21]

All calculations were carried out on an IBM 370 computer using the CRYM system of crystallographic programs developed by David J. Duchamp. Atomic form factors are from "International Tables for X-Ray Crystallography",[22] except for H, which was taken from Stewart, Davidson, and Simpson.[23] The final agreement index ($R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$) was 0.030. The standard deviation of fit = $[\Sigma w(|F_o|^2 - |F_c|^2)^2/(m - s)]^{1/2}$ was 4.07. The final value of the secondary extinction parameter g was 3.4(2) × 10^{-5}.

[3a*S*-(3aα,5α,6α,6aα)]-(1,2-diacetyloxyethyl)-1,3,3a,4,5,6a-hexahydro-6-(acetyloxy)-3-methyl-2*H*-furo[2,3-*d*]imidazol-2-one (6). A solution of 386 mg of **5** in 10 mL of $(CH_3CO)_2O$-pyr (1:1) was allowed to stand at room temperature for 24 h. It was then added slowly with stirring to 40 mL of cold H_2O. The aqueous solution was extracted with three 10-mL portions of ethyl acetate. The combined extracts were washed with 10 mL of H_2O, 10 mL of 0.5 N HCl, 10 mL of saturated $NaHCO_3$, and 10 mL of H_2O, dried ($MgSO_4$), and evaporated to dryness in vacuo. The residue was chromatographed on 16 g of silica gel using $CHCl_3$–CH_3OH (98:2), collecting 2.5-mL fractions. On the basis of a weight analysis, fractions 16–29 were combined and evaporated to dryness in vacuo to give 261 mg of product: TLC (SiO_2; $CHCl_3$–CH_3OH, 95:5) showed only one spot, R_f 0.61; mp 100–102 °C; IR (Nujol) 3225, 1730 (broad), 1695, 1235, 1015, 945, 875, and 750 cm^{-1}; ^1H NMR ($CDCl_3$) 2.01, 2.06 (2 s, 9 H, $3CH_3COO$), 2.85 (s, 3 H, CH_3N), 4.06 (d, 1 H, *J* = 0, 6.5 Hz, H-2), 4.16 (d of d, 1 H, *J* = 5.5, 12.0 Hz, H-6β), 4.17 (d of d, 1 H, *J* = 3.0, 9.0 Hz, H-4), 4.52 (d of d, 1 H, *J* = 2.3, 12.0 Hz, H-6α), 5.18 (d of d, 1 H, *J* = 0, 3.0 Hz, H-3), 5.22 (m, 1 H, *J* = 9.0, 2.3, 5.2 Hz, H-5), 5.55 (d, 1 H, *J* = 6.5 Hz, anomeric H); ^{13}C NMR ($CDCl_3$) 170.4, 169.7, 169.5 (3 CH_3CO), 159.6 (NCON), 91.3 (C-1, 75.9, 75.3 (C-4 and C-5), 67.6 (C-3), 63.1 (C-6), 59.5 (C-2, 27.3 (CH_3N), 20.5 ($3CH_3CO$); mass spectrum *m/e* 344.1218 (M^+) (calcd for $C_{14}H_{20}N_2O_8$, 344.1220).

Anal. Calcd for $C_{14}H_{20}N_2O_8$: C, 48.84; H, 5.85; N, 8.13. Found: C, 48.12; H, 6.07; N, 7.05.

5-[(*E*)-4-Hydroxy-2-butenylidene]-3-methyl-2,4-imidazolidinedione (7). A. From 1. A solution of 1.0 g of **1** in 25 mL of 1 M H_2NSO_3H was allowed to stand at room temperature for 24 h. The crystalline precipitate (200 mg) was removed by filtration and re-

crystallized from H_2O: yield 170 mg of orange prisms; mp 215–217 °C; pK_a' 10.6 (CH_3OH–H_2O; 6:4); UV (CH_3OH) 245 nm (ϵ 9100), 310 (ϵ 32 396), 320 sh; IR (Nujol) 3440, 3200, 1765, 1700, 1655, 1030, and 980 cm^{-1}; ^1H NMR (pyridine-d_5) 3.08 (s, 3 H, CH_3N), 4.50 (d of d, 2 H, J = 3.9, 6.5 Hz, CH_2), 5.62 (broad s, 1 H, exch H), 6.38 (d of t, 1 H, J = 3.9, 14.9 Hz, H-5), 6.54 (d, 1 H, J = 12.0 Hz, H-3), 7.18 (m, 1 H, J = 12.0, 14.9 Hz, H-4), 10.20 (br s, 1 H, enolic H); ^{13}C NMR (Me_2SO-d_6) 163.7 (NC=O), 154.3 [N=C(OH)N], 141.6 (C-4), 127.3 (C-2), 122.5 (C-5), 110.0 (C-3), 61.2 (C-6), 24.1 (CH_3N); mass spectrum m/e 182 (calcd for $C_8H_{10}N_2O_3$, 182).

Anal. Calcd for $C_8H_{10}N_2O_3$: C, 52.79; H, 5.54; N, 15.39; O, 26.37. Found: C, 52.48; H, 5.73; N, 15.21; O, 26.64.

B. From 5. This was run as in A using 100 mg of starting material; yield 40 mg. Identified by comparison of melting point and infrared and NMR spectra with those of material obtained in A.

Alkaline Degradation of 7. A solution of 800 mg of **7** in 50 mL of 1 N NaOH was refluxed for 2.5 h. During the hydrolysis N_2 was passed through the solution and into 10 mL of 1 N HCl. Potentiometric titration of an aliquot indicated that 0.9 mol of volatile base per mole of **7** had been trapped. An aliquot of the acid solution was evaporated to dryness, and the residue was crystallized from CH_3OH–$(C_2H_5)_2O$. The precipitate was identified as CH_3NH_2·HCl by ^1H NMR spectroscopy.

Another aliquot of the acid solution was added to 250 mL of Brady's reagent. The crystalline precipitate was removed and recrystallized from 95% EtOH. It was identified as acetaldehyde 2,4-dinitrophenylhydrazone by melting point, UV, IR, and analysis.

An alkaline hydrolysate similar to the above was acidified with 19 mL of 6 N HCl, and N_2 was passed through the solution and on into a $Ba(OH)_2$ solution. The precipitate of $BaCO_3$ was removed by filtration, washed, and dried. It indicated formation of 0.6 mol of CO_2 per mole of **7**. The remaining acidified solution was added to an excess of Brady's reagent. The resulting precipitate was removed and identified as glyoxal bis(2,4-dinitrophenylhydrazone) by comparing its UV and IR spectra to those of an authentic sample.

[4*R*,5*R*(1*S*,2*R*)]-4-Acetyl-5-(1,2,3-trihydroxypropyl)-2-oxazolidinone (9). **1** (1 g) was dissolved in 5 mL of Me_2SO, and the solution was allowed to stand for 18 h at room temperature. The solvent was removed by evaporation in a stream of air, leaving a clear syrup. This was chromatographed on 100 g of silica gel using the solvent system $CH_3COC_2H_5$–CH_3COCH_3–H_2O (73:23:5) and collecting 5-mL fractions. On the basis of a weight analysis, fractions 61–102 were combined, and the solution was evaporated in vacuo to an aqueous residue which was freeze dried; weight 322 mg of amorphous solid. The residue was dissolved in water, the solution was filtered, and the filtrate was freeze-dried: yield 238 mg; R_f 0.45 (SiO_2; $CH_3COC_2H_5$–CH_3COCH_3–H_2O, 70:20:11); IR (neat) 3305, 2905, 1750, 1410, 1225, 1085, 1020, 995, 880, 820, and 760 cm^{-1}; ^1H NMR (D_2O) 2.38 (s, 3 H, CH_3CO); ^{13}C NMR (Me_2SO-d_6) 209.6 (CH_3**C**O) 160.0 (**N**COO), 78.2 (C-5), 73.2 (C-3), 72.3 (C-4), 65.0 (C-6), 63.0 (C-2), 28.0 (**C**H_3CO); mass spectrum [as 4$(CH_3)_3Si$] m/e 492.2072 ($M^+ - CH_3$) (calcd for $C_{19}H_{42}NO_6Si_4$, 492.2089).

Anal. Calcd for $C_8H_{13}NO_6$: C, 43.83; H, 5.98; N, 6.39. Found: C, 41.54; H, 6.06; N, 5.81.

[4*R*,5*R*(1*S*,2*R*)]-3,4-Diacetyl-5-(1,2,3-triacetyloxypropyl)-2-oxazolidinone (10). Crude **9** (1 g) was acetylated as described under the preparation of **6**, yield 0.84 g. Chromatography on 100 g of silica gel in Skellysolve B–acetone (6:4) and collecting 5-mL fractions gave two weight maxima after weight analysis. Fractions 55–75 were combined and evaporated in vacuo, yield 350 mg. Two recrystallizations from EtOH gave 186 mg: mp 83–85 °C; R_f 0.61 (SiO_2; Skellysolve B–acetone, 1:1); $[\alpha]_D$ −36.5° (c 1, EtOH); IR (Nujol) 1785, 1755, 1730, 1690, 1290, 1205, 1125, 1025, 960, 915, 835, and 750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.06, 2.15, 2.36, 2.58 (4 s, 15 H, 5CH_3CO), 4.19 (d of d, 1 H, J = 4.3, 12.5 Hz, H-6α), 4.35 (d of d, 1 H, J = 2.6, 12.5 Hz, H-6β), 4.63 (d of d, 1 H, J = 3.4, 2.4 Hz, H-3), 4.69 (d, 1 H, J = 3.4 Hz, H-2), 5.26 (m, 1 H, J = 7.8, 2.6, 4.3 Hz, H-5), 5.36 (d of d, 1 H, J = 2.4, 7.8 Hz, H-4); ^{13}C NMR ($CDCl_3$) 201.8 (CH_3**C**O), 170.4, 169.9, 169.7, 169.5 (CH_3**C**OO, CH_3**C**ON), 73.1 (C-5), 70.0 (C-3), 69.1 (C-4), 62.1 (C-6), 61.2 (C-2), 27.5 (**C**H_3COC), 22.9 20.7, 20.6, 20.3 (3**C**H_3COO, **C**H_3CON); mass spectrum (FD) m/e 387 (M^+) (calcd for $C_{16}H_{21}NO_{10}$, 387).

Anal. Calcd for $C_{16}H_{21}NO_{10}$: C, 49.61; H, 5.47; N, 3.62. Found: C, 49.66; H, 5.45; N, 3.31.

A crystalline 2,4-dinitrophenylhydrazone was formed from **10** using the procedure of Shriner and Fuson.[24]

[1*R*,6*R*,5*R*,5a*R*,10*R*,10a*R*(1*S*,2*R*)]-5,10-Bis(acetyloxypropyl)-3*H*,8*H*-bisoxazolo[3,4-*a*:3′,4′-*d*]pyrazine-3,8-dione (11). Saline solution (80 mL, 0.9%) was adjusted to pH 4.6 with 0.05 M citric acid. **1** (5 g) was added. In a few minutes foaming occurred, and there was a rapid rise in pH to ~8.5. The solution was concentrated in vacuo to a viscous liquid; weight 4.5 g. This material was dissolved in a mixture of 10 mL of $(CH_3CO)_2O$ and 100 mL of dry pyridine and allowed to stand at room temperature for 24 h. The volatile material was removed by evaporation in vacuo, and the residue was dissolved in 100 mL of $CHCl_3$. The resulting solution was washed with 100 mL of 2 N HCl, 40 mL of 1 N HCl, two 40-mL portions of saturated $NaHCO_3$, and two 40-mL portions of H_2O. It was then dried ($MgSO_4$) and evaporated to dryness in vacuo; weight 3.75 g. The residue was combined with 1.3 g of similarly prepared material and chromatographed on 960 g of silica gel in $CHCl_3$–CH_3OH (98:2) collecting 20-mL fractions. A weight analysis indicated three weight peaks. The two slower moving weight maxima were worked up, but were not obtained sufficiently pure for identification. Fractions 210–220 were combined and evaporated under reduced pressure to give 0.61 g of residue. The material was purified by three successive solutions in warm ethanol and cooling. In the first case the precipitate was amorphous, in the second it was partially crystalline, and in the third completely crystalline: yield 131 mg; mp 180–182 °C; R_f 0.69 (SiO_2; $CHCl_3$–CH_3OH, 92.5:7.5); $[\alpha]^{25}_D$ −90° (c 1, CH_3COCH_3); IR (Nujol) 1790, 1745, 1220, 1110, 1085, 1070, 1050, 1020, 960, 925, 865, and 755 cm^{-1}; ^1H NMR ($CDCl_3$) 2.09, 2.14, 2.16, 2.17 (4 s, 24 H, 8 CH_3CO), 3.59 (d of d, 2 H, J = 6.0, 4.0 Hz, H-2 and 2′), 4.32 (s, 4 H, H-6 and 6′), 5.19 (d, 2 H, J = 4.0 Hz, H-3 and 3′), 5.33 (s, 4 H, H-4 and 4′ and H-5 and 5′), 6.13 (d, 2 H, J = 6.0 Hz, anomeric H); ^{13}C NMR (Me_2SO-d_6) 170.3 (6 CH_3**C**OO), 169.5 (2 CH_3**C**OO), 154.2 (2 NCO), 79.3 (2 anomeric), 77.1, 75.2 69.0 (C-3,4,5 and C-3′,4′,5′), 61.4 (C-6 and 6′), 55.7 (C-2 and 2′), 20.6 (8**C**H_3CO); mass spectrum m/e 686.18154 ($M^+ - CH_3COOH$) (calcd for $C_{28}H_{34}N_2O_{18}$, 686.1806).

Anal. Calcd for $C_{30}H_{38}N_2O_{20}$: C, 48.26; H, 5.13; N, 3.76. Found: C, 48.24; H, 5.10; N, 4.03.

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Registry No.—**1**, 18883-66-4; **2**, 27321-18-2; **3**, 68107-80-2; **4**, 68050,71-5; **5**, 68050-72-6; **6**, 68050-73-7; **7**, 53428-56-1; **9**, 68050-74-8; **10**, 68050-75-9; **11**, 68050-76-0; glucosamine hydrochloride, 66-84-2.

References and Notes

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(2) Streptozocin [2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose] was formerly known as streptozotocin. Numbering in all compounds except **11** is not as indicated in the Experimental Section titles, but is the same as that of the corresponding carbon in **1**. In the case of **11**, one half is numbered in this fashion, while the other half is designated C-1′, C-2′, etc. The numbering system used in the crystallography studies differs again and is indicated in Figure 1.
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Syntheses and Chiroptical Properties of (+)-2-Brendanone and Its Analogues[1]

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Syntheses of (+)-2-brendanone (**12**), (+)-2-norbrendanone (**14**), and (−)-2-isobrendanone (**16**) from the respective optically active bicyclic intermediates (**38**, **37**, and **54**) with known absolute configurations establish their absolute configurations. As the lowest homologue of 2-brendanone, (+)-tricyclo[2.2.2.$0^{2,6}$]octan-3-one (**18**) was prepared by the oxidative decarboxylation of (−)-*endo*-5-bicyclo[2.2.2]octene-2-carboxylic acid (**43**). Absolute rotations and chiroptical properties of these 2-brendanone analogues are discussed.

Simultaneous bridging C-2 to C-5 and C-3 to C-6 positions of cyclohexane with short carbon chains ($m,n \leq 2$) freezes the cyclohexane moiety in a twist-boat conformation (Chart I) to yield the rigid tricyclic hydrocarbons (**1**) which have D_2 symmetry when $m = n = 2$, C_2 symmetry when $m \neq n$, and D_{2d} symmetry when $m = n = 1$. (−)-Twistane (**3**) (D_2 symmetry)[2] and (−)-*twist*-brendane (**5**) (C_2 symmetry)[3] can be cited as the typical representatives of these gyrochiral[4] cage-shaped hydrocarbons whose syntheses and absolute configurations have been reported from our laboratory. Starting from cyclohexanone, the same diagonal C-2 to C-5 and C-3 to C-6 bridgings afford tricyclic ketones with lower symmetries, among which (−)-2-twistanone (**4**) (C_2 symmetry),[2] (−)-2-*twist*-brendanone (**6**) (C_1 symmetry),[3] and (−)-2-bis(noradamantanone) (**8**) (C_2 symmetry)[5] have been synthesized with known absolute configurations. In contrast with these doubly diagonal bridging, simultaneous C-2 to C-4 and C-3 to C-6 bridgings (Chart II) fix the cyclohexane ring in a boat conformation giving achiral tricyclic hydrocarbons (**9**) all belonging to the C_s point group as can be seen in brendane (**11**),[6] norbrendane (**13**),[7] isobrendane (**15**),[7] and tricyclo[2.2.2.$0^{2,6}$]octane (**17**).[9] Introduction of carbonyl group breaks the bilateral symmetry inherent to these brendane analogue cage-shaped hydrocarbons to provide the tricyclic ketones **12**, **14**, **16**, and **18** with cyclohexanone moieties frozen in a boat conformation. Natural extention of our current interest in syntheses and chiroptical properties[10] as well as microbial stereodifferentiating reduction[11] of the cage-shaped tricyclic ketones led us to investigate the preparation of these types of tricyclic ketones in optically active modification from the intermediates with known absolute configurations.

Chart I

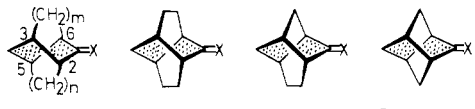
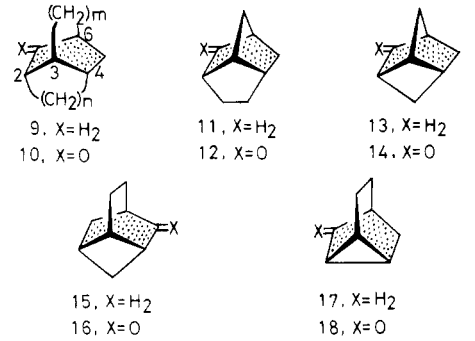


Chart II



Results and Discussion

Since previous synthetic routes for both 2-brendanone (**12**)[6] and 2-norbrendanone (**14**)[8] appeared inconvenient either for obtaining them in optically active modification or for correlating them with the intermediates with known absolute configurations, we designed the sequence of steps encompassed in Scheme I in which the bicyclic endo-keto ester (**27**) is a strategic intermediate which correlates (−)-(1*S*,2*S*,4*S*)-*endo*-5-bicyclo[2.2.1]heptene-2-carboxylic acid (**19**) with the bicyclic keto mesylates **37** and **38** whose intramolecular alkylation should lead to optically active 2-brendanone (**12**) and 2-norbrendanone (**14**), respectively.

Preparation of (−)-(1*S*,2*S*,4*S*)-*endo*-2-Carbomethoxybicyclo[2.2.1]heptan-5-one (27) (Scheme I). Spurlock's procedure[12] reported for the racemic compounds was applied with a slight modification to the conversion of the (−)-unsaturated carboxylic acid (**19**) into the optically active keto ester (**27**). A mixture of the hydroxy esters **21** obtained by hydroboration–oxidation of (−)-(1*S*,2*S*,4*S*)-*endo*-2-carbomethoxybicyclo[2.2.1]hept-5-ene (**20**), $[\alpha]^{16}_{D}$ −91.1° (optical purity 67%),[13] was treated with Jones' reagent to afford a 2:3 mixture of 5- and 6-keto esters **22** whose sodium borohydride reduction gave a mixture of 5- and 6-*endo*-hydroxy esters **23**. Separation of these regioisomers was carried out by saponification of the mixture of methyl esters followed by acidification which, while converting the 6-endo isomer into the (−)-lactone (**24**), mp 153.5–155 °C, $[\alpha]D^{15}_{D}$ −2.8° (EtOH), left the 5-endo isomer as the free 5-*endo*-hydroxycarboxylic acid (**25**). The separated hydroxy acid **25** was converted into methyl ester **26** whose Corey's oxidation yielded (−)-(1*S*,2*S*,4*S*)-keto ester **27**, $[\alpha]^{15}_{D}$ −21.3° (EtOH).

Synthesis of (+)-2-Norbrendanone (14) [(+)-Tricyclo[3.2.1.$0^{3,6}$]octan-2-one] (Scheme I). Continuation of the synthesis would involve (1) modification of the carboxyl group to give the keto mesylate (**37**) and (2) its intramolecular al-

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