SYNTHESIS, CHARACTERIZATION, AND CONFORMATION IN SOLU-TION OF A NOVEL D-GLUCURONO-6,1-LACTAM DERIVATIVE

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ABSTRACT

Prolonged treatment of 2'-(sodium β -D-glucopyranosyluronate)isonicotinohydrazide with acetic anhydride in the presence of pyridine at room temperature for ~110 h gave 2,3,4-tri-O-acetyl-N-(diacetylamino)- β -D-glucopyranurono-1,6-lactam (2), a novel D-glucurono-6,1-lactam derivative. Its conformation in solution, examined by ¹H- and ¹³C-n.m.r. and compared with the crystal structure, corresponds to a fairly rigid ring system, the pyranose ring adopting a near $B_{0,3}$ (D) boat form in solution and a distorted ¹C₄ (D) chair conformation in the crystalline state. The closely related 2,3,4-tri-O-acetyl- β -D-glucopyranurono-6,1-lactone (4) and 2,3,4-tri-O-acetyl-1,6-anhydro- β -D-glucose (3) take the distorted ¹C₄ (D) chair conformation, both in solution and in crystal form. Based on the crystal structure data of 4, the parameters of the Karplus-type equation, which relates vicinal-coupling constants with dihedral angles, were determined for 2, 3, and 4.

INTRODUCTION

Structures of 1,6-anhydrohexopyranoses and their related compounds have been extensively studied¹⁻¹⁴. The results from X-ray diffraction strongly support the conclusion that the pyranose ring has the ${}^{1}C_{4}$ (D) chair conformation in crystals with more or less distortion depending²⁻⁵ on the configuration and the nature of the substituents at C-2, -3, and -4. The pyranose ring of 1,6-anhydro- β -D-glucose and its triacetate is distorted from the ideal ${}^{1}C_{4}$ (D) chair to the ${}^{3}E_{0}$ (D) form so as to reduce the nonbonding repulsion of diaxial substituents, because the pyranose ring is strained by the formation of the anhydro ring²⁻⁴. The ${}^{1}C_{4}$ (D) chair conformation in solution has been verified for 1,6-anhydrohexopyranoses and related compounds

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by the close agreement between the results of n.m.r. spectroscopy⁶⁻¹⁴ and those of the optical rotation^{15,16}. Recently, an n.m.r. study of 3-amino-1,6-anhydro-3-deoxy- β -D-glucopyranose (1,6-anhydrokanosamine) and of its hydrochloride has been reported, indicating that the pyranose ring of the former compound in solution is in rapid equilibrium between the ¹C₄ (D) chair and B_{0,3} (D) boat forms, whereas that of the latter compound is only the boat form¹⁷. On the other hand, the distorted ¹C₄ (D) chair conformation has been verified for both compounds in the crystalline state^{18,19}. It is of interest that the behavior of the ring conformations in solid state and solution would depend only on the replacement of OH-3 by NH₂-3. This behavior of the structure has been interpreted in terms of intramolecular hydrogen-bonding and interaction with the solvent^{17,18}.

In the course of our investigation of ring-chain interconversions of sugar hydrazides and hydrazones in solution²⁰, we have synthesized, from 2'-(sodium β -D-glucopyranosyluronate) isonicotinohydrazide (1), a D-glucosylurono-6,1-lactam derivative, 2.3.4-tri-O-acetyl-N-(diacetylamino)- β -D-glucopyranurono-1,6-lactam (2), that has a new ring system. Its chemical structure was determined by several analytical and spectroscopic techniques. The results from ¹H- and ¹³C-n.m.r. support a near $B_{0,3}$ (D) boat conformation and a fairly rigid nature of the ring skeleton in solution, whereas a distorted ${}^{1}C_{+}$ (D) chair conformation has been verified in the crystalline state by X-ray crystal-structure analysis²¹. As this compound has no hydrogen atom in the molecule involved in intramolecular hydrogen-bonding, and as it has the fused five-membered ring, whose C-6 methylene group in 1,6-anhydro sugars is replaced by a carbonyl group, it may be assumed that factors exist which take part in the conformational preference and are different from those reported for 1,6-anhydrokanosamine¹⁷. In order to obtain more information about these factors, the molecular structure and conformation in solution of 2,3,4-tri-O-acetyl-β-D-glucopyranurono-6,1-lactone²² (4), a compound closely related to 2, was examined. The conformation of 2 and i in solution was compared with that of 2,3,4-tri-O-acetyl-1,6-anhydro-B-Dglucose^{4,10} (3).



The present paper reports the synthesis, characterization, and conformation in solution of 2, and the conformation in solution of 4. The detailed crystal structures of 2 and 4 will be reported separately²¹. Steric hindrance of the conformation in solution of 2, 3, and 4 was examined by molecular model building by an iterative, computer method, and the empirical parameters of the Karplus-type equation²³⁻²⁵ for 2, 3, and 4 were examined by a computer program also described in this paper.

RESULTS AND DISCUSSION

Synthesis and structure elucidation of 2. — Compound 2 was synthesized by treating 1 with a large excess amount of acetic anhydride in the presence of pyridine at room temperature, followed by processing as usual. The mol. wt. was estimated to be 400 by the osmotic vapor pressure method. The e. i. mass spectrum shows no parent peak observable around m/z 400, and the peaks at m/z 358 and 316, probably resulting from the loss of acetyl fragments from the molecular ion with a hydrogen transfer, or from elimination of ketene from the molecular ion²⁶, indicate also a mol. wt. of 400. The elemental analysis suggests the presence of two nitrogen atoms in the molecule.

A comparison of the i.r. spectra of 1 and 2 showed, in the spectrum of 2, (a) full acetylation of the amino and hydroxyl groups (disappearance of peaks due to the N-H and the O-H stretching vibrations and appearance of strong peaks assignable to the C=O and C-O stretching vibrations, and the C-H deformation vibrations of the acetyl group); (b) the presence of a γ -lactone or formation of a bridge involving the C-O carboxyl group (disappearance of bands due to the carboxylate ion, and appearance of new bands assignable to the C=O and the C-O stretching vibrations of a γ -lactone, but not of a δ -lactone); and (c) lack of the isonicotinoyl residue (disappearance of bands at 3030, 1600, 753, and 705 cm⁻¹ which were assigned to the aromatic C-H and C=C stretching vibrations and to the monosubstituted, aromatic C-H, out-of-plane deformation vibrations, respectively; and disappearance of peaks at ~1660 and 1550 cm⁻¹, which were assigned to the C=O stretching and the N-H deformation vibrations of the amide group).

The disappearance, in the u.v. spectrum of 2, of absorption bands at 264 nm (molar absorptivity of 6500), which is assignable to the isonicotinoyl residue of 1, and appearance of a band having a maximum absorption at a wavelength shorter than 230 nm also support the absence of the isonicotinoyl residue.

The 200-MHz¹H-n.m.r. spectrum of 2 for a solution in 2:1 (v/v) chloroform-dmethanol-d₄ shows no ¹H-resonance exchangeable with deuterium, supporting full substitution of the hydroxyl and amino groups, which is in agreement with the results from the i.r. spectrum. No appearance of ¹H-resonances in the aromatic region indicated absence of the isonicotinoyl residue. These observations ruled out the possibilities of 2 being the acetyl derivative of 1, as was expected from the investigation on aldohexose isonicotinohydrazides²⁷, or an oxadiazoline derivative of 1, as has been reported for aldohexose 4-acetamidobenzoylhydrazones²⁸. The positions

Coupling constants	Compound						
(Hz)	2	3	4				
3J _{1.2}	0.6	1.2	1.5				
³ J _{2,3}	5.1	1.7	1.5				
³ J _{3.4}	5.5	1.7	2.0				
³ J _{4.5}	1.2	(2.2)*	2.0				
${}^{3}J_{5,6}$		1.0					
³ J _{5.6} ,		5.5					
² J _{6,6} ,		7.6					
⁴ J _{1,3}	0.5	1.2	1.5				
$4J_{2,4}$	0	1.7	1.5				
⁴ J _{3,5}	0.6	1.7	2.0				
4J _{1.5}	0	0	0				
⁴ J _{1.6}		0.3					
⁴ J _{1,6} ,		0.4					
$5J_{2.5}$		(0.5) ^b	0.5				

VICINAL AND LONG-RANGE COUPLING CONSTANTS FOR 2, 3, AND 4^{α}

^aDetermined for solutions in solvent *III* at 200 MHz. ^bData from refs. 8 and 14, which were taken because of unresolved ¹H-resonances for H-4 and -5 of 3.

of two broad singlets at δ 2.58 and 2.36 ruled out the possibilities of acetoxyl^{14,29,30}. methoxyl^{30,31}, and acetylamino³² groups. Absence of ¹H-resonances exchangeable with deuterium indicated the tertiary nature of the two nitrogen atoms. The presence of an N.N-dimethyl group was excluded, as methyl protons of the N.N-dimethyl group in desosamine appear³³ as a sharp singlet at δ 2.75. The resonance positions and the broad nature of these singlets are suggestive of the existence of a hindered rotation around the methyl protons. Indeed, temperature dependence of the n.m.r. spectrum of 2 in dimethyl sulfoxide- d_6 solution, in the temperature range of 25–105°, indicated the existence of a hindered rotation about the N-C bond: the two broad singlets coalesced at 45-50°, and the broad peak became gradually sharp with increasing temperature. The resonance position of the peak was observed at the center of the two singlets. This result suggests the presence of an N,N-diacetyl group, but not of a tertiary N,N'-diacetyl group, as the two methyl protons of the former group should become a singlet at a temperature higher than the coalescence temperature, whereas those of the latter group may experience a nonequivalent chemical environment, which causes the two methyl singlets to appear as separate signals. Thus, the two broad signals were assigned to the methyl protons of the N,N-diacetyl group. Besides the signals just described, only five other well-resolved signals appeared, which were assigned to the ring protons of the sugar skeleton. The ${}^{1}H^{-1}H$ vicinal coupling constants (<5.5 Hz, Table I) indicate that 2 has a conformation that is different from the ${}^{4}C_{1}$ (D) chair conformation of the parent compound, as vicinal coupling constants of the β -D-gluco anomer having the ${}^{4}C_{1}$ (D) chair conformation



Fig. 1. 200-MHz ¹H-n.m.r. spectrum of the ring protons of 2 for a solution in 2:1 (v/v) chloroform-d-methanol- d_4 (solvent *III*).

are known²⁹ to be ~ 8 Hz. In addition, the spectral data for the ring protons that permit the first-order analysis also rule out the possibility of 2 being the β -D anomer in the ${}^{4}C_{1}$ (D) chair form 34,35 . The possibility of 2 having a 6,3-lactone (y-lactone) ring was excluded, as the ¹H-n.m.r. spectrum showed the presence of three acetoxyl groups and one N, N-diacetyl group. The five-membered 6, 1-lactam ring was established by the presence, in the ¹H-n.m.r. spectrum (see Fig. 1), of a multiplet, appearing in the lowest field at δ 5.39, that was assigned to H-3 by double-resonance experiments and by comparison of reported assignments for peracetyl sugar derivatives^{29,34,36}; this signal was found to be coupled with all the remaining signals, and particularly with the two signals at δ 5.12 and 4.98, by large coupling constants, 5.5 and 5.1 Hz, respectively. As the presence of three acetoxyl groups attached to the sugar ring is evident, the ring proton nuclei bearing the acetoxyl groups should be deshielded^{29,36} and, hence, the three ¹H-resonances that are coupled with each other may be assigned to H-2, -3, and -4. The signal at δ 4.55 appearing in the highest field among the ring protons was assigned to H-5 ($J_{3,5}$ 0.6 Hz), and that appearing at δ 5.12 to H-4 $(J_{4.5} \text{ 1.2 Hz})$. A broad singlet at δ 5.27 was assigned to H-1, and the only remaining signal at δ 4.98 to H-2. These assignments indicate the presence of a pyranose ring. Furthermore, the values reported for the vicinal coupling-constants ${}^{3}J_{2,3}$ and ${}^{3}J_{3,4}$, for methyl- β -D-xylo-5-hexulofuranosidurono-6,3-lactone (0 and 5 Hz, respectively)³⁷, also exclude the possibility of 2 having a furanose ring.

The only structure that satisfies all the experimental results is that of 2,3,4-tri-O-acetyl-N-(diacetylamino)- β -D-glucopyranurono-1,6-lactam (2). This structure was confirmed by X-ray crystal structure analysis²¹. The analysis indicated that the 6,1lactam ring is in the envelope form, and that the pyranose ring has the distorted ${}^{1}C_{4}$ (D) chair conformation. Compound 2 is, to our knowledge, a novel D-glucurono-6,1-lactam derivative having a hitherto unknown ring system that resembles that of

TABLE II

Atoms	Com	Compound										
	2	2			3			4				
	Ι	II	III	IV	I	II	III	IV	I	II	III	IV
H-1	5.15	5.17	5.270	5.76	5.37	5.47	5.460	5.41	5.90	5.96	5,963	6.21
H-2	4.95	4.98	4.993	4.97	4.52	4.64	4.586	4.66	4.77	4.80	4,791	4.73
H-3	5.33	5.39	5.387	5.23	4.77	4.86	4.842	4.66	4.93	4.94	4.956	4.85
H-4	5.02	5.14	5.125	4.93	4.60	4.64	4.66	4.66	4.77	4.81	4.820	4.74
H-5	4.50	4.55	4.578	4.88	4.56	4.64	4.64	4.66	4.57	4.64	4.639	4.92
H-6					4.06	4.10	4.122	4.09				
H-6'					3.72	3.82	3.819	3.67				
OCOCH₃	2.10	2.14	2.153	2.06	2.12	2.18	2.19	2.09	2.16	2.18	2.207	2.13
	2.08	2.13	2.140	2.05	2.09	2.15	2.16	2.07	2.16	2.18	2.207	2.13
	2.03	2.08	2.096	2.02	2.07	2.12	2.13	2.07	2.09	2.11	2.125	2.04
N(COCH ₃)	2.50	2.54	2.578	2.48								
	2.32	2.36	2.360	2.29								
	Solve	nt shif	7Þ									
	I–II	III	-IV I	-IV	I–II	<i>III</i> -	-IV I-	IV	I–II	III–	IV I-1	v
H-1	-0.0	2 -(0.49 -	-0.61	-0.10	0	.05 —	0.04	-0.06	-0	25 -(. 31
H-2	-0.0	3 (0.02 -	-0.02	-0.12	0	07 -	0 14	-0.03	0	06 (04
H-3	-0.0	6 (0.16	0.10	-0.09	Ō	.18	0.11	-0.01	Ő.	11 (0.08
H-4	-0.1	2 (0.20	0.09	-0.04	0	· –	0.06	-0.04	0	08 (0.03
H-5	-0.0	5 -0	0.30 -	-0.38	-0.08	-0	.02 -	0.10	-0.07	-0	28 -0	0.35

¹H-CHEMICAL SHIFTS FOR COMPOUNDS 2, 3, AND 4 IN VARIOUS SOLVENTS^a

^{a1}H-Chemical shifts for solutions in dichloromethane- $d_2(I)$, chloroform-d(II), and dimethyl sulfoxide $d_6(IV)$, were recorded at 100 MHz, and those for solution in 2:1 (v/v), chloroform-d-methanol- d_4 at 200-MHz ¹H-n.m.r.; δ from Me₄Si signal. ^bA negative sign denotes a down-field shift, and a positive sign an up-field shift.

1,6-anhydro sugars. However, the values of ${}^{3}J_{2,3}$ and ${}^{3}J_{3,4}$ for 2 significantly differ from those reported for 1,6-anhydro- β -D-glucose⁸, its triacetate (3)¹⁰, and trimethyl ether⁹, suggesting a different conformation in solution.

Conformation of 2 in solution. — The chemical shifts and tentative assignments of ¹H-resonances for 2, 3, and 4 were studied for solutions in 2:1 (v/v) chloroform-dmethanol- d_4 at 200 MHz, and in various solvents at 100 MHz (see Table II). Splitting patterns of ¹H-resonances for all the ring protons, except those of H-4 and -5 of 3, allowed first-order analysis of the 200-MHz, ¹H-n.m.r. spectra, but, for the 100-MHz spectra, resonance patterns for H-2, -4, and -5 of 3, and for H-2 and -4 of 4 were complicated by signal overlapping and their second-order nature. A notable feature is the peak for H-3 of 2. This signal appears at a frequency higher than that of H-1 by δ 0.12, and than those for H-3 of 3 and 4 by δ 0.54 and 0.43, respectively, indicating

a somewhat different ring geometry centered at C-3. It should be noted that the H-5 signals of 2, 3, and 4 appear at nearly the same positions, irrespective of the different substituents on the fused five-membered rings, whereas the chemical shifts of the H-1 signals are spread for the three compounds. Thus, the substituents on the five-membered ring affect more the H-1 than the H-5 chemical shift. Study of the solvent effect on ¹H-chemical shifts of the ring protons shows that H-1 and -5 signals of 2 and 4were greatly affected by the change of solvent from 2:1 (v/v) chloroform-d-methanol d_4 to dimethyl sulfoxide- d_6 , and little by the change to dichloromethane- d_7 or chloroform-d. The signals for H-1 and -5 of 3, and H-2, -3, and -4 of 2, 3, and 4 were little affected by changes of the solvent. This may be interpreted by assuming that dimethyl sulfoxide- d_6 comes in closer contact with H-1 and -5 than with H-2, -3, and -4 protons, owing to the steric interaction arising from the diaxial acetoxyl groups, and may interact with the ring protons as a proton-accepting solvent. Thus, H-5 of 2 and 4, which are active methine protons, and H-1 of 2 and 4, which have an acidic character, would take part in hydrogen bonding with the solvent, resulting in substantial downfield shifts of the ¹H-resonances. The most pronounced down-field shift observed for H-1 of 2 may be due to the stronger acidic character of H-1, as compared to the other protons, and the relatively small solvent-effect observed for H-1 and -5 of 3 may be ascribed to the different substituent at C-6.

The vicinal $({}^{3}J_{i,i})$ and long-range $({}^{4}J_{i,k}$ and ${}^{5}J_{i,l})$ coupling constants for 2, 3, and 4 were obtained by measurements of signal spacings in the 200-MHz ¹H-n.m.r. spectra (see Table I). The values for 3 were in close agreement with those reported earlier^{10,12}, and with those reported for 1,6-anhydro- β -D-glucose as well⁸, within 0.3 Hz. As the ${}^{3}J_{4,5}$ value of 3 could not be obtained with accuracy owing to the second-order nature of the signals, a value of 2.2 Hz was taken from the literature⁸. The values of ${}^{3}J_{i,j}$ for 3 and 4 lie in a narrow range (1.2–2.0 Hz), whereas those for 2 do not. The same order of magnitude for ${}^{3}J_{i,j}$ as ${}^{4}J_{i,k}$ values was observed for 3 and 4, whereas much smaller values of ${}^{4}J_{i,k}$ were observed for 2. These results suggest that 3 and 4 have a similar conformation in solution where a ${}^{1}H_{-}{}^{1}H$ coplanarity is fairly retained, whereas 2 has a different conformation in solution. The solvent effect on ${}^{3}J_{i,i}$ was examined for 2, 3, and 4 in chloroform-d, 2:1 (v/v) chloroform-dmethanol- d_4 , and dimethyl sulfoxide- d_6 . The values ${}^3J_{i,j}$ for 2, 3, and 4 were almost invariable ($< \text{ or } = \pm 0.2 \text{ Hz}$ variations, data not shown), indicating that the ring conformations of 2, 3, and 4 are largely independent of the polarity of the solvent. This observation is in good agreement with the small changes of the ¹H-chemical shifts of H-2, -3, and -4 caused by the changes of solvent, as presented earlier.

The chemical shifts and tentative assignments of the ¹³C-resonances in the ¹³C-n.m.r. spectra at 25.1 MHz for 2, 3, and 4 in chloroform-d and dimethyl sulfoxided₆ solution are given in Table III. The assignments of the ¹³C-resonances for the pyranose-ring carbon atoms were obtained by use of partial-proton-decoupling and proton-selective-decoupling techniques, together with comparisons with literature data^{38,39}. The reported assignments for 3 are in good agreement with those obtained in our experiment, except for the C-2 and -3 resonances, which were found to be

TABLE III

Atom	Compound						
	2		3 (1)	4 (I)			
	I	II					
C-1	90.46	89.64	99.55	100.53			
C-2	70.48	70.86	69.78	65.88			
C-3	71.20	71.82	70.12	68.95			
C-4	69.26	69.89	70.85	66.08			
C-5	76.88	76.10	74.17	71.15			
CH2-6			65.69				
C = 0	167.35	167.06	169.82	167.92			
	169.44	169.10	170.41	168.55			
	170.32	169.29	170.65	169.29			
		169.63		169.39			
		171.38					
OCOCH ₃	20.73	20.57	20.86	20.61			
N(COCH ₃) ₂	24.46	25.33					
		23.78					

¹³C-CHEMICAL SHIFTS AND TENTATIVE ASSIGNMENTS FOR COMPOUNDS 2, 3, AND 4^a

^aDetermined at 25.1 MHz in proton-noise-decoupled mode for solutions in chloroform-*d* (*I*), and for solutions in dimethyl sulfoxide- d_6 (*II*); δ from Me₄Si signal. Assignments for nonquarternary carbon atoms were achieved by the use of partial proton-decoupling and proton-selective decoupling techniques together with literature data^{38,39}. ¹³C-Signals for quarternary carbons were not assigned.

reversed. Unambiguous assignments for the quarternary carbons have not been established, owing to signal overlapping and their long relaxation times. Of the ¹³Cresonances corresponding to the ring carbon atoms of 2, 3, and 4, those of C-1 appear at the lowest field, and those of C-3 at a field higher than those of C-1 and -5, indicating that the ¹³C-chemical shifts of the pyranose-ring carbon atoms may be less sensitive to the ring geometry than the ¹H-chemical shifts. Solvent effect on ¹³Cchemical shifts was little observed for 2, the resonances ascribable to the two methyl carbons of the *N*,*N*-diacetyl group appearing as two singlets for dimethyl sulfoxide-*d*₆, and as a broad signal for chloroform-*d* solution. As the resonance position of the signal is at the center of the two singlets, the existence of a hindered rotation is evident also from the ¹³C-n.m.r. signals. In addition, they reveal that the N–C bonds may experience in dimethyl sulfoxide-*d*₆ solution a rotational barrier larger than that in chloroform-*d* solution.

Temperature dependency of ¹H-chemical shifts, coupling constants, and spectral linewidths of the spectra for 2, 3, and 4 gave information on the ring puckering of the compounds in solution, as the conformations of 1,6-anhydro sugars are strongly restricted and conformational transition is centered²⁹ at C-3. In the chair-to-boat transition, the geometry of H-1 and -5 is almost unchanged in contrast to that of H-2, -3, and -4. Thus, the magnitudes of the change of dihedral angles in the

pyranose ring for H-1 and -2 ($\psi_{1,2}$) and H-4 and -5 ($\psi_{4,5}$) is smaller than for H-2 and -3 ($\psi_{2,3}$), and H-3 and -4 ($\psi_{3,4}$). The ¹H-n.m.r. spectra recorded at 100 MHz and +21 to -89° for solutions in dichloromethane- d_2 , and +21 to -60° for solutions in 2:1 (v/v) chloroform-d-methanol- d_4 showed a negligible change, with decreasing temperature, in the linewidths of the peaks of ring protons for 2, 3, and 4, indicating no scope for signal coalescence. Furthermore, no peak that reflects conformational "freeze out" and no significant change in the vicinal coupling-constants with decreasing temperature was observed. Down-field shifts of ¹H-resonances (δ 0.04–0.16) were observed for 2, 3, and 4. Of the ring protons, H-2, -3, and -4 exhibited smaller down-field shifts (δ 0.04–0.10) with decreasing temperature from +21° to -89°. In addition, the 13 C-n.m.r. spectra of 3 for a solution in chloroform-d, which were recorded at several temperatures, showed small up-field shifts ($\delta \sim 0.7$) for C-2, -3, and -4 with decreasing temperature from $+30^{\circ}$ to -60° . A coalescence temperature of -68° and an energy barrier for ring puckering of $42 \pm 1 \text{ kJ} \cdot \text{mol}^{-1}$ have been reported for 1,2,3,4-tetra-O-acetyl- β -D-ribopyranose from a temperature, ¹H-n.m.r. study⁴⁰ at 100-MHz. But the absence of signal coalescence of significant changes in ¹H- and ¹³C-chemical shifts and coupling constants with decreasing temperature, and the solvent independence of 1 H-chemical shifts and coupling constants for 2, 3,

TABLE IV

Atoms	Compound 2	3	4	Atoms	Compound 3 (ref. 4)
H-1, H-2				H-1, H-2	
J_{1}^{0}	0.6	1.2	1.5	$J_{1,2}^{c}$	1.6
ψ_{1}^{c}	-72.1	-66.4	-64.0	w ⁰	
•1.2	-107.9	-113.4	-116.0	• 1,=	-63.1
H-2, H-3				H-2, H-3	
J_2^0	5.1	1.7	1.5	J_{a}^{c}	0
ψ_{a}^{c}	40.4	68.0	64.0	w ⁰	86.6
• 2.3	139.6	112.0	116.0	1 2.3	
H-3, H-4				H-3. H-4	
$J_{3,4}^0$	5.5	1.7	2.0	J_{2}^{c} .	0
$\psi_{\mathbf{x},\mathbf{z}}^{\mathbf{c}}$	-38.5	-68.0	-60.3	ν ⁰	85.4
	-141.5	-112.0	-119.7	, 3, 4	
H-4. H-5				H-4. H-5	
J_{A}^{0}	1.2	2.2°	2.0	J_{A5}^{c}	2.3
$\psi_{4,5}^{e}$	66.4	58.9	60.3	$\psi_{1,5}^{\dagger}$	58.2
- 1,4	113.6	121.1	119.7	. 4.0	

CONFORMATIONAL PARAMETERS FOR HYDROGEN ATOMS ON THE PYRANOSE RING OF COMPOUNDS $\mathbf{2}, \mathbf{3}$, and $\mathbf{4}^{\mathfrak{g}}$

^aValues for $J_{i,i}^{0}$ (Hz) were the observed vicinal-coupling constants; values for $\psi_{i,j}^{c}$ (degrees), dihedral angle, were obtained by application of the Karplus-type equation²³⁻²⁵ to $J_{i,j}^{0}$; signs of $\psi_{i,j}^{c}$ correspond to those of internal-rotation angles ($\psi_{i,j}^{0}$) from the crystal-structure data for 3 (ref. 4); and values for $J_{i,j}^{c}$ are the calculated, vicinal-coupling constants (Hz); ^bref. 8.

and 4 just discussed suggests that the three compounds have fairly rigid skeletons in solution within the experimental temperature range. Chair-boat transition for 1,6-anhydro- β -D-glucose in crystal form at high temperature has been observed by i.r. spectroscopy⁴¹, but such a transition was not observed by ¹H-n.m.r. spectroscopy⁴². Examination of the solution of 2 in dimethyl sulfoxide- d_6 at high temperature by ¹H-n.m.r. spectroscopy showed a slight increase in magnitude (~0.5 Hz) of ${}^{3}J_{2,3}$ and ${}^{3}J_{3,4}$ values with increasing temperature from +25 to 105°. This increase, however, corresponds to an increase in dihedral angle of ~5° according to the Karplus equation. Consequently, a chair-boat transition did not occur in the solution up to 105°.

Observed vicinal coupling constants $(J_{i,i}^{o})$ for 2, 3, and 4 from the ¹H-n.m.r. spectra, and calculated dihedral angles $(\psi_{i,i}^c)$, obtained by application of the Karplus type equations, $J = 9.26 \cos^2 \psi - 0.28$ (Ref. 24) and $J = 10.5 \cos^2 \psi - 1.2 \cos \psi$ (Ref. 25), lead to near equal values (see Table IV). Two roots of $\psi_{i,i}$ for $0^{\circ} \leq \psi_{i,i}$ $\leq 90^{\circ}$, and $90^{\circ} \leq \psi_{i,i} \leq 180^{\circ}$ were obtained from $J_{i,i}$. The signs of $\psi_{i,i}^{\circ}$ correspond to those of $\psi_{i,i}^{o}$ reported earlier⁴. A comparison of $J_{i,i}^{o}$ with $J_{i,j}^{c}$ for 3 reveals good agreement for $J_{1,2}$ and $J_{4,5}$, indicating that the conformational difference between crystal and solution depends on H-2, -3, and -4. The values of $J_{i,i}^{o}$ for 2, 3, and 4 show almost equal magnitudes for $J_{1,2}^{o}$ and $J_{4,5}^{o}$, and $J_{2,3}^{o}$ and $J_{3,4}^{o}$, respectively, leading to the relations $\psi_{1,2} \approx \psi_{4,5}$ and $\psi_{2,3} \approx \psi_{3,4}$. Nearly identical values were obtained from the results of the X-ray analysis^{4,21} for 2, 3, and 4, $\psi_{1,2}^c$ (and $\psi_{2,2}^c$) being nearly equal to $\psi_{2,3}^{c}$ (and $\psi_{3,4}^{c}$) values for 3 and 4, indicating that the coupled protons are in gauche or eclipsed relationship. The value of $\psi_{2,3}^{c} \sim 65^{\circ}$ for 3 and 4 correspond to an almost ideal ${}^{1}C_{4}$ (D) chair form, and $\sim 115^{\circ}$ for 3 and 4 to a near ${}^{3}E_{0}$ (D) sofa form. However, the possibility of 3 and 4 taking up the ideal ${}^{1}C_{4}$ (D) chair conformation in solution may be ruled out because of the nonbonding repulsion of atoms of the three axial acetoxyl groups $^{2-4}$. The possibility of the latter conformation may also be excluded as the values of $\psi_{1,2}^c$, $\psi_{2,3}^c$, $\psi_{3,4}^c$, and $\psi_{4,5}^c$ cannot be the same value of 115° owing to the conformationally locked ring-system. The value of $\psi_{2,3}^{c}$ (and $\psi_{3,4}^{c}$) ~40° for 2 is improbable because of the large steric hindrance and nonbonding repulsion of the axial acetoxyl groups. The value of $\psi_{2,3}^c \sim 140^\circ$, which corresponds to the near-boat form, is less probable, as the value of $\psi_{1,2}^{c}$ (and $\psi_{4,2}^{c}$) $\sim 110^{\circ}$ is larger that that measured from a Dreiding molecular model. Therefore, any value of $\psi_{i,i}^c$, for 2, 3, and 4 presented in Table IV, would lead to conformations exhibiting steric hindrance, nonbonding repulsion, or unreasonable internal rotation angles. In support of this conclusion, we built a molecular model, with the intention of minimizing as much as possible the deviations of all the ψ_{i}^{c} , bond angles, and bond distances of the sugar ring.

Computer model. — The building of a computer model was based on the method of Dodson *et al.*⁴³, which minimizes bond-distance deviation, by adding more parameters (bond angle and internal rotation angle). By setting the values of $\psi_{2,3}^c$ and $\psi_{3,4}^c$ at ~40° for 2, and at ~65° for 3 and 4, we obtained two models, but both of them showed abnormality in bond distances (>0.01 nm deviations from the initial

values), nonbonded distances (>0.06 nm deviations from the initial values), and bond angles (>10° deviations from the initial values) for some atoms. Molecular models built by setting the values of $\psi_{2,3}^{e}$ and $\psi_{3,4}^{e}$ at ~140° for 2, and at ~115° for 3 and 4 gave abnormal bond-lengths for C-1–O-5, and C-5–O-5. The results of molecular model building indicate that no molecular model (as shown in Table IV) exists that can optimize all $\psi_{i,j}^{e}$, bond-distance and bond-angle values at the same time. Thus, the parameters for the Karplus type equation (A and B in $J : A \cos^2 \psi - B$), may be unsuitable for application of the equation to such conformationally locked ringsystems as found in 2, 3, and 4.

To determine the empirical parameters for the equation on the basis of the X-ray structure data for 4, we gave a value to $\psi_{2,3}$ and $\psi_{3,4}$, and, based on the computer program described in the Experimental section, the values of $\psi_{1,2}$ and $\psi_{4,5}$ could be obtained by adopting the values of bond lengths and bond angles for 4. Change of the values of $\psi_{2,3}$ and $\psi_{3,4}$, and assumption of $\psi_{2,3} = \psi_{3,4}$ afforded the corresponding values $\psi_{1,2}$ and $\psi_{4,5}$. By plotting $\psi_{1,2}$ vs. $\psi_{2,3}$, a linear relationship, $\psi_{1,2} = 0.5 \psi_{2,3} + 17.0$, was derived. The mean value of $J_{2,3}^{o}$ and $J_{3,4}^{o}$ was employed as $J_{2,3}^{o'}$, and $J_{4,5}^{o}$ as $J_{4,5}^{o'}$, in order to determine the parameters A and B for 2, 3, and 4. The parameters A and B are related to $J_{2,3}^{o'}$, $J_{4,5}^{o'}$, $\psi_{2,3}^{c}$, and $\psi_{4,5}^{c}$, as shown in the coalition equations (1)-(3).

$$J_{2,3}^{o'} = A \cos^2 \psi_{2,3}^c + B \tag{1}$$

$$J_{4,5}^{o'} = A \cos^2 \psi_{4,5}^{c} + B \tag{2}$$

$$\psi_{4,5}^{c'} = 0.5 \,\psi_{2,3}^{c} + 17.0 \tag{3}$$

Five variables; A, B, and $\psi_{2,3}^{c}$ for 2, 3, and 4, were changed for six observations, so as to minimize the R value in $R = \Sigma (J_{i,j}^{o'} - J_{i,j}^{c})^2$. Actually, the parameters A and B, and the value $\psi_{2,3}^{c}$ were changed alternately to stabilize the least-squares method. Initially, values of A 14,00 Hz; B 0 Hz; and $\psi_{2,3}^{c}$ 95.0°, 90.0°, and 90.0° for 2, 3, and 4, respectively, were selected. After twenty iterations, the refined variables of A 7.12 Hz; B 1.21 Hz; $\psi_{2,3}^{c'}$ 139.1°, 104.1°, and 106.2°, for 2, 3, and 4 respectively, were obtained with an R value of 0.02. When the values A 14.00 Hz; B 0 Hz; and $\psi_{2,3}^{\circ}$ 115.0°, 110.0°, and 10.0°, respectively, were selected initially, the refined values of A 15.22 Hz; B 0.46 Hz; and $\psi_{2,3}^{c'}$ 124.9°, 106.5°, and 107.3°, for 2, 3, and 4, respectively, were obtained with an R value of 0.10. For justifying the refined values, we gave values of $\psi_{2,3}^{c'}$, $J_{2,3}^{o'}$, and $J_{4,5}^{o'}$ to the above described equations (1)-(3), and calculated A and B for the three compounds, respectively. Change of $\psi_{2,3}^{c}$ from 85° to 155° for every 2.5° afforded the corresponding A and B values. Fig. 2 shows A and B (Hz) vs. $\psi_{2,3}^{c'}$ for 2, 3, and 4 in the range 85–155°. The ranges of A and B, which appear common for the three compounds, were \sim 7-16 Hz and 0.5-1.5 Hz, respectively. For the ranges of A and B, the $\psi_{2,3}^{c'}$ value for 2 is in the range 125-140°, and that of $\psi_{2,3}^{c'}$ for 3 and 4 in the range $104-107^{\circ}$. The value for A is distributed over a wide range, whereas that for B exhibits a positive sign and is in a narrow range. The value for $\psi_{2,3}^{c'}$ for 2 is distributed over a wide range dependent upon the value of A and B,



Fig. 2. Plot of A and B (Hz) versus $\psi_{2,3}^{c'}$ (degrees) for 2 (--×-), 3 (---), and 4 (--O--). Areas indicated by shaded lines show the ranges of the values of A and B that appear common for 2, 3, and 4.

whereas that of $\psi_{2,3}^{c'}$ for 3 and 4 is almost invariant with A and B. The evidence that, of the two values for $\psi_{2,3}^{c'}$, 139.1° or 124.9°, the former reflects the true conformation of 2 is based on the excellent agreement with the dihedral angle obtained by application of the Karplus type equation to $J_{2,3}^{o}$ (see Table IV), and the observation that the R value for the former is smaller than that for the latter value. Thus, the empirical parameters for A and B were determined to be 7.12 and 1.21 Hz, respectively, for 2, 3, and 4.

TABLE V

Atoms	Compound			
	2	3	4	
H-2, H-3	······································			
$J_{2,3}^{0'}$	5.30	1.70	1.72	
$\Psi_{2,3}^{e}$	139.1	104.1	106.2	
$J_{2,3}^{c}$	5.2s	1.63	1.75	
H-4, H-5				
$J_{4.5}^{0'}$	1.2	2.2	2.0	
$\psi_{4.5}^{e^{\psi}}$	86.6	69.0	70.1	
$J_{4,5}^{c^{*}}$	1.22	2.21	2.0_{2}	

CONFORMATIONAL PARAMETERS FOR HYDROGEN ATOMS ON THE PYRANOSE RING OF COMPOUNDS 2, 3, and 4 calculated by an iterative computer-method^a

^aThe computer program used is described in the Experimental section; $J_{2,3}^{0^c}$, (Hz) are the mean values of $J_{2,3}^{0}$ and $J_{3,4}^{0}$; $\psi_{1,1}^{c^c}$, (degrees) the dihedral angles obtained by use of the least-squares method; and $J_{i,1}^{c^c}$ (Hz) the calculated vicinal-coupling constants by introducing $\psi_{i,j}^{c}$ into the equations (1)-(3).

The values for $\psi_{2,3}^{c'}$ and $\psi_{4,5}^{c'}$ for 2, 3, and 4 were compared to the corresponding $J_{2,3}^{c'}$, $J_{4,5}^{c}$, $J_{2,3}^{o}$, and $J_{4,5}^{c}$ values (see Table V). Values for $\psi_{4,5}^{c'}$ of 86.2°, 69.0°, and 70.1° were obtained for 2, 3, and 4, respectively. The $J_{i,j}^{c'}$ values for 2, 3, and 4 are all in excellent agreement with $J_{i,j}^{o}$ (see Table V). A comparison of the value of $\psi_{2,3}^{c'}$ with that of $\psi_{2,3}^{c}$ (see Table IV) reveals a good agreement within $\pm 10^{\circ}$, whereas poor agreement is observed between $\psi_{4,5}^{c'}$ and $\psi_{4,5}^{c}$. This suggests that, in such conformationally locked ring-system, the Karplus equation²³⁻²⁵ is applicable to a hydrogen atom of the pyranose ring whose puckering motion is allowed, but is not applicable where such motion is highly restricted.

The plot of A and B vs. $\psi_{2,3}^{e'}$ (see Fig. 2) suggests a near $B_{0,3}$ (D) boat form for 2 and a distorted ${}^{1}C_{4}$ (D) chair form for 3 and 4. A comparison of $\psi_{2,3}^{e'}$ of 3 with $\psi_{2,3}^{o}$ from crystal structure data reveals that the pyranose ring is more distorted from the ${}^{1}C_{4}$ (D) chair to the ${}^{3}E_{0}$ (D) sofa form in solution than in crystal form. The differences of the ring conformation of 2 between crystal and solution appears to be much more pronounced. In order to ascertain the postulated conformation in solution for 2, 3, and 4, the building of molecular models that can optimize bonding informations, *i.e.*, $\psi_{i,j}^{e'}$, bond distance, and bond angle, at the same time was attempted (see Fig. 3). The models exhibit no abnormality in nonbonded distance, indicating no serious steric hindrance. The observation that 2 exhibits long-range coupling constants smaller than 3 and 4 (see Table II) is illustrated by use of the molecular models (Fig. 3). The molecular model for 3 and 4 having $\psi_{2,3}^{e'}$ of 105° involves several bonds in the pyranose ring, which hold near W-conformation⁴⁴⁻⁴⁶. For example, in the bonds of H-1-C-1-C-2-C-3-H-3, the distances of H-1 and H-3 from the least squares plane defined by C-1, -2, and -3 were calculated to be 0.019 and 0.072 nm,



Fig. 3. Perspective views of molecular models for 2 (left), and 3 and 4 (right) in solution. The models were drawn by computation for $\psi \underline{s}_{,3}^{*}$ of 140[°] (left) and 105[°] (right).

respectively. In contrast the model for 2 having a value $\psi_{2,3}^{c}$ of 140° involves no bonds that retain a W-conformation at all, probably resulting in substantially smaller long-range coupling constants.

In conclusion, the postulated conformations for 2, 3, and 4 in solution agree with the steric hindrance observed in molecular-model building. The results from n.m.r. spectroscopy suggest that 2, a novel D-glucurono-6,1-lactam derivative, possesses a fairly rigid ring-skeleton in solution, and its pyranose ring adopts a near $B_{0,3}$ (D) boat form, whereas 3 and 4 have a distorted ${}^{1}C_{4}$ (D) chair form. The observation that the pyranose ring of 3 and 4 adopts to the same extent a flattening at C-3 in solution indicates that replacement of CH_2 -6 of 3 by a carbonyl group may little affect the conformation in solution. The observation that the pyranose ring of 2 adopts the near-boat form in solution, whereas it adopts the distorted ${}^{1}C_{4}$ (D) chair form in crystalline state²¹, may be interpreted in terms of either steric, electronic, or solvation effect. The steric effect of the N,N-diacetyl group on AcO-3, however, may not be so serious as the nitrogen atom of the 6,1-lactam ring has sp^3 character, and the other nitrogen atom linked to the two acetyl groups has sp^2 character. Hence, the nonbonded distances of the atoms belonging to the AcO-3 and the N,N-diacetyl groups do not become shorter than 0.337 nm (data not shown) by rotating the N,Ndiacetyl group around the N-N bond from 0° to 360°, and by calculating the nonbonded distances of the atoms at every 10° of rotation. The ¹H-n.m.r. study of solvent effect on the ¹H-chemical shifts and coupling constants of the ring protons for 2 showed no appreciable change of the ring conformation with the polarity of solvent, suggesting little contribution of solvation. Therefore, distortion of electronic distribution due to the presence of the 6,1-lactam ring that bears the N,N-diacetyl group may cause the pyranose ring to adopt the near $B_{0,3}$ (D) boat form in solution. However, no evidence to support this assumption exists at this stage, and a comparative study of the ring conformation of 2, 3, and 4 by molecular mechanics will be necessary.

EXPERIMENTAL

General. - Optical rotations were measured for chloroform solutions at the D line in 1-dm cells with a Perkin-Elmer 241 polarimeter equipped with a thermo bath. Direct-probe mass spectra were equipped with a Shimazu LKB 9000 Gas chromatography-Mass spectrometer at room temperature, the ionizing voltage being 70 eV. U.v. spectra for ethanol solutions were recorded with a Hitachi 624 Digital Spectrometer, and i.r. spectra for potassium bromide discs with a Hitachi 285 instrument. ¹H-N.m.r. spectra were recorded for solutions in dichloromethane-d₂ (solvent I), chloroform-d (solvent II), 2:1 (v/v), chloroform-d-methanol- d_4 (solvent III), and dimethyl sulfoxide- d_6 (solvent IV) with JNM FX-200 and JNM FX-100 instruments, in the pulse, Fourier-transform NMR system, and with a JNM PS-100 instrument in the continuous wave-mode. ¹H-Chemical shifts were determined, relative to an internal standard of tetramethylsilane, by data reduction. Coupling constants (Hz) were determined from the apparent, first-order analysis with the aid of homonuclear, spin-decoupling experiments. Temperature dependence of n.m.r. spectra were measured at 21° to -89° for solutions in dichloromethane- d_2 , and at 21° to -60° for solutions in 2:1 (v/v) chloroform-d-methanol-d₁. ¹³C-N.m.r. spectra were recorded for solutions in chloroform-d (solvent I) and dimethyl sulfoxide- d_6 (solvent II), with a JNM FX-100 instrument in the pulse, Fourier-transform mode locked on deuterium. ¹³C-Chemical shifts were recorded, relative to the protondecoupled ¹³C resonance of tetramethylsilane, by data reduction. ¹³C-N.m.r. spectra at different temperatures were obtained for solutions of 3 in chloroform-d.

Computer model building. — In equation (4), the quantity E was minimized by the least-squares procedure.

$$E = W_l (l_{i,j}^o - l_{i,j}^c)^2 + W_{\theta} (\theta_{i,j,k}^o - \theta_{i,j,k}^c)^2 + W_{\psi} (\psi_{i,j,k,l}^o - \psi_{i,j,k,l}^c)^2$$
(4)

 $l_{i,j}$ is the bond distance (or sometimes nonbonded distance) between atoms i and j; $\theta_{i,j,k}$ is the bond angle defined by atoms i, j, and k; and $\psi_{i,j,k,l}$ is the internal rotationangle defined by atoms i, j, k, and l. The superscripts o and c indicate a given and a calculated value, respectively, which the model has in the current cycle of least squares. The values for W_l , W_{θ} , and W_{ψ} are weights for the least-squares fitting, which should be the force constants of quadratic terms physically. In this minimization process, the terms higher than quadratic were neglected. To build the model as strictly as possible in every state of the pyranose-ring puckering, the force constants of higher terms might have to be known, because the compound has a large steric-restraint in every state. However, for application of the Karplus-type equation, it is sufficient to use equation (4) and equation (5): each parameter, l, θ , ψ , can be expanded by x_i , y_i , and z_i of Cartesian coordinates of atom i. In (5), parameter p represents one of the parameters of l, (θ and ψ), and w is equal to one of the weights of W_l (W_{θ} and W_{ψ}) of equation (4). The values $\partial p/\partial x_i$, $\partial p/\partial y_i$, are taken from Boyd⁴⁷.

$$w \frac{\partial p}{\partial x_i} \Delta x_i + w \frac{\partial p}{\partial y_i} \Delta y_i + \dots + w \frac{\partial p}{\partial y_s} \Delta y_s + w \frac{\partial p}{\partial z_s} \Delta z_s = w(p^\circ - p^\circ) \quad (5)$$

Equation (5) can be expressed by use of a matrix and column vector as: $\tilde{A}\tilde{x} = \tilde{h}$ (6), where \tilde{A} is the asymmetric sparse-matrix, \tilde{h} the residual vector, and \tilde{x} the displacement vector. Dodson et al.⁴³ added, in equation (7), quantity Q to energy terms in order to avoid instability in the least squares,

$$Q = \Sigma w' \{ (x_i^{\circ} - x_i^{\circ})^2 + (y_i^{\circ} - y_i^{\circ})^2 + (z_i^{\circ} - z_i^{\circ})^2 \}$$
(7)

the superscript o representing the target. In our study, the target coordinates are always the calculated coordinates in every cycle of least squares, that is, $x_i^o = x_i^c$. In the model building, the values of x, y, z, l, and θ of compound 4, which were obtained by X-ray structure analysis, were the observed ones. By application of the conjugategradient method, scaling of the matrix elements gave convergence⁴³.

2,3,4-Tri-O-acetyl-N-(diacetylamino)- β -D-glucopyranurono-1,6-lactam (2). — To a solution of 2'-(sodium β -D-glucopyranosyluronate)isonicotinohydrazide²⁰ (1) (2.0 g) in dry pyridine (25 mL) was added acetic anhydride (15 mL). The solution was kept with stirring at room temperature for ~110 h, until almost all of 1 was dissolved. The dark-red solution was filtered, and ethanol (10 mL) was added. The solution was evaporated *in vacuo* with subsequent addition of ice-water to give a yellow-brown precipitate. The precipitate was filtered off and crystallized from ethanol (40 mL) to give 2 as colorless prisms (37% yield), m.p. 183°, $[\alpha]_D^{20} - 37.5°$ (*c* 2, chloroform); v_{max}^{KBr} 1775 (C=O str. 1,4-lactone), 1754, 1742, 1730 (C=O str. OAc), 1365 (C-H def. OAc), 1242, 1233, 1225 (C-O str. OAc), 1200 cm⁻¹ (C-O str. 1,4-lactone); ¹H-n.m.r. (solvent *III*): δ 5.39 (m, H-3), 5.27 (broad s, anomeric H-1), 5.12 (dd, H-4), 4.98 (dd, H-2), 4.55 (m, H-5). 2.58, 2.36 [broad s, N(COCH₃)] 2.15, 2.14, and 2.10 (s, 3 OCOCH₃)^{29.30}, see also Fig. 2, and Tables I and II; ¹³Cn.m.r. data, see Table III; *m/z*: 358 (M⁺ -42), 316 (M⁺ -85), 85 (Ac · OCCH₂), and 43 (CH₃-CO⁺).

Anal. Calc. for C₁₆H₂₀N₂O₁₀: C, 48.12; H, 4.92; N, 6.97. Found: C, 48.12; H, 4.80; N, 7.01.

2,3,4-Tri-O-acetyl-1,6-anhydro- β -D-glucopyranose^{48,49} (3). — This compound was prepared from phenyl β -D-glucopyranoside by alkaline hydrolysis to yield 1,6anhydro- β -D-glucose, followed by acetylation with acetic anhydride and by crystallization from ether to give 3 as colorless prisms (60% yield), m.p. 108°, $[\alpha]_D^{20} - 58.0^\circ$ (c 2, chloroform); lit.⁴⁹ m.p. 107–110°, $[\alpha]_D^{25} - 61.5^\circ$ (c 1, chloroform).

2,3,4-Tri-O-acetyl- β -D-glucopyranurono-6,1-lactone²² (4). — This compound was prepared by acetylation of sodium D-glucopyranuronate with acetic anhydride in the presence of *p*-toluenesulfonic acid to give a mixture of α - and β -D- anomers of 1,2,3,4-tetra-O-acetyl-D-glucopyranuronic acid, followed by isolation of the β -D anomer by fractional precipitation. The β -D anomer was then dehydrated with stannic chloride, and crystallization from ethanol gave 4 as colorless prisms (10% yield), m.p. 127°, $[\alpha]_D^{20}$ -66.0° (c 2, chloroform); lit.²² m.p. 122.5-124°, $[\alpha]_D^{19}$ -78.6° (c 0.9, chloroform).

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