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CONFORMATIONS OF THE D-GLUCAROLACTONES AND D-GLUCARIC ACID IN SOLUTION*†

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ABSTRACT

The conformations of D-glucaric acid (**1**), D-glucaro-1,4-lactone (**2**), D-glucaro-6,3-lactone (**3**), and D-glucaro-1,4:6,3-dilactone (**4**) in solution were investigated by ^{1}H-n.m.r. and ^{13}C-p.F.t., n.m.r. spectroscopy. The solvents used were deuterium oxide, methanol-d_4, and dimethyl sulfoxide-d_6, and praseodymium chloride was employed as a lanthanide shift-reagent. For **2**, it was found that the conformational equilibrium ^3E(D) ⇄ E_3(D) exists in solution, and that the OH-5 group tends to occupy the position over the lactone ring in the favored E_3(D),*gg* conformation. The n.m.r. data for **3** indicated that the conformational equilibrium is shifted in favor of the ^4E(D) ⇄ E_4(D),*gt* conformation in solution. The dienvelope conformation ^3E:E_4(D) was found to be the favored conformation of **4**. For **1**, a conformational equilibrium between one planar, zigzag form and two sickle forms was indicated by the n.m.r. data observed. ^{13}C-N.m.r. spectroscopy proved to be a convenient method for monitoring the lactonization of **1**, and the hydrolysis of its lactones. Lactones other than **2**–**4** were not found in solutions prepared from **1**–**4**, either during their mutarotation or after equilibration at 30°.

INTRODUCTION

D-Glucaric acid (**1**), D-glucaro-1,4-lactone (**2**), D-glucaro-6,3-lactone (**3**), and D-glucaro-1,4:6,3-dilactone (**4**) are compounds of significant biological interest. They are normal metabolites involved in the metabolism of D-glucuronic acid in mammals[2]; lactone **2** is a powerful, competitive and highly specific inhibitor of β-D-glucosiduronase[3,4], and **4** is a precursor of **2**. The production of **2** from D-glucuronic acid can probably modify β-D-glucosiduronase levels in the body. Lactone **2** has been considered as a potential drug for treating diseases in which it is suspected that there may be a disorder of D-glucuronic acid metabolism. Compound **2** and, later, its sodium salt[5,6], as well as **4** and its 2,5-di-*O*-acetyl derivative[7], have been used in the

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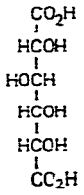
treatment of bladder cancer. The 2,5-di-*O*-acetyl derivative of **4** and the sodium salt of **2** have also been used in the treatment of rheumatoid arthritis[8]. It was found that **2** has anticoagulant properties[9], and that, among D-glucaric acid derivatives, lactone **2** has the greatest lysosome-stabilizing activity[10]. Evidence has been submitted that **2** shortens the pharmacological action of drugs being disposed of, *via* the bile, as D-glucosiduronates[11]. It has also been shown that derivatives of both **2** and **4** can decrease the side effects of such aminocyclitol antibiotics as kanamycin[12].



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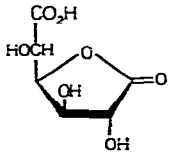

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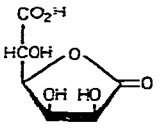

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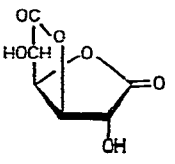

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Our studies on the conformations of the D-glucarolactones and D-glucaric acid in solution were undertaken in order to provide conformational data that might be helpful in explaining the behavior of these compounds in biological systems, and that might be of general interest for conformational analysis of sugar acid lactones. Preliminary studies on the conformations of the D-glucarolactones in solution[13–15] made use of i.r.- and u.v.-spectroscopic, and optical rotatory, methods; ^{1}H-n.m.r. spectroscopy at 60 MHz was also employed in order to furnish detailed conformational information, but only in the case of **4** were first-order spectra observed[13,14]. In the present work, ^{1}H-n.m.r. measurements were made at 100 MHz for solutions in deuterium oxide, methanol-d_4, or dimethyl sulfoxide-d_6, including measurements in the presence of praseodymium chloride as a shift reagent, to give first-order spectra of all four compounds (**1–4**). A detailed analysis of the spectra allowed determination of the conformations of **1–4** in solution. The results have been confirmed by proton-decoupled, natural-abundance-carbon-13, pulse Fourier-transform (p.F.t.), n.m.r. spectroscopy for solutions of **1–4** in deuterium oxide and in dimethyl sulfoxide-d_6.

RESULTS AND DISCUSSION

General. — Because of resonance considerations, 1,4-lactones would be expected to adopt envelope conformations. X-Ray diffraction studies have verified this supposition for a number of crystalline, non-sugar 1,4-lactones, and also for three aldono-1,4-lactones[16–18] and, more recently[19], for **2**. Likewise, for 1,4-lactones in solution, partial double-bond character within the ring should prevent the pseudorotation that must be considered for saturated, five-membered rings, and so envelope conformations are also to be anticipated for 1,4-lactones in solution[20–22]. In envelope conformations, the carbonyl group of the 1,4-lactone ring lies in the favored, staggered disposition in relation to the nonbonding orbitals of the ring-oxygen atom as a

consequence of the[23] "gauche effect"; an alternative, twist conformation wherein the carbonyl group is eclipsed with one of the nonbonding orbitals of the ring-oxygen atom would be less stable. Electrostatic repulsion between the dipoles of the nonbonding orbitals of the oxygen atom and the dipole of the carbonyl group may be expected to constitute a strong destabilizing factor for lactones[24]. The length of the C-1–O-4 bond in crystalline **2** was found[19] to be 133 pm, whereas that of the carbon–oxygen double bond is predicted to be 127 pm, and that of the carbon–oxygen single bond is 143 pm. The value of 135 pm found for the C-1–O-4 bond in the three aldono-1,4-lactones studied[16–18] is also close to that predicted for the carbon–oxygen double bond. All of these facts indicate that envelope conformations should also be favored in solution.

Therefore, the following n.m.r. data found for **2** and **3** were interpreted in terms of envelope conformations, and conformational equilibria between two alternative envelope forms were considered. For compound **4**, a dienvelope conformation, and, for **1**, an equilibrium of sickle conformations, as well as the planar, zigzag conformation, were considered. Although resonances of individual protons generally appeared in very narrow ranges in the ^{1}H-n.m.r. spectra of **1–4**, it was, by changing solvents or using lanthanide shift-reagents, found possible to obtain spectra that were quite well resolved and were amenable to complete, first-order analysis. As a further test of ^{1}H-n.m.r. chemical-shifts and coupling constants, computer-simulated spectra were generated. Conformational data deduced from the ^{1}H-n.m.r. spectra were then confirmed by ^{13}C-n.m.r. measurements. Assignments were made according to general rules that have been applied with 1,4-lactones[21,22,25,26], together with others developed for furanoid sugars[27–29]. The assignments are in good agreement with data found for the aldono-1,4-lactones[30]. Differences in chemical shifts measured for solutions

TABLE I

100-MHz, ^{1}H-N.M.R.-SPECTRAL DATA FOR **1–4**

Compound	*Solvent*	*Chemical shifts (d values[a])*				*Coupling constants (Hz)*			
		H-2	*H-3*	*H-4*	*H-5*	$^3J_{2,3}$	$^3J_{3,4}$	$^3J_{4,5}$	$^4J_{2,4}$
1	D_2O	4 82d	4.49dd	4.32dd	4.71d	3.0	5.5	5.0	—
	methanol-d_4	4.38d	4.15dd	3.97dd	4.26d	3.0	4.5	5.5	—
2	D_2O	5.11d	4.94dd	5.47dd	4.99d	8.9	7.8	2.8	—
	D_2O[b]	4.48d	4.87dd	5.63dd	5.42d	8.8	7.8	2.8	—
	methanol-d_4	4.72d	4.53m	5.04dd	4.52m	8.8	7.8	2.8	—
3	D_2O[b]	5.52d	5.32dd	5.17dd	5.10d	6.7	2.5	4.6	—
	Me_2SO-d_6[c]	4.67m	4.90m	4.72m	4.93m	8.0	3.0	4.8	—
4	D_2O	4.98q	5.57dd	5.90o	5.35d	0.8	3.9	5.4	0.4
	methanol-d_4	4.32q	4.95dd	5.29o	4.76d	0.7	3.6	5.0	0.4

[a]Signal multiplicities: d, doublet; m, complex multiplet; o, octet. [b]In the presence of 0.04 mol. equiv. of $PrCl_3$. [c]In the presence of CF_3CO_2H.

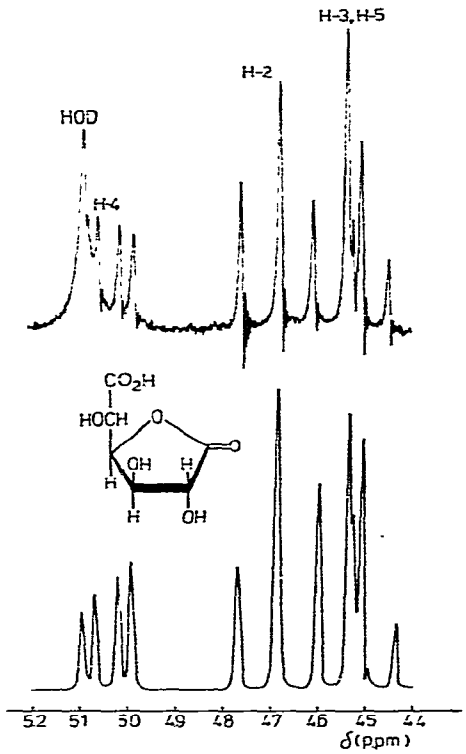


Fig. 1. The 100-MHz, ^{1}H-n.m.r. spectrum of D-glucaro-1,4-lactone (2) in methanol-d_4 (upper trace), and the simulated spectrum (lower trace).

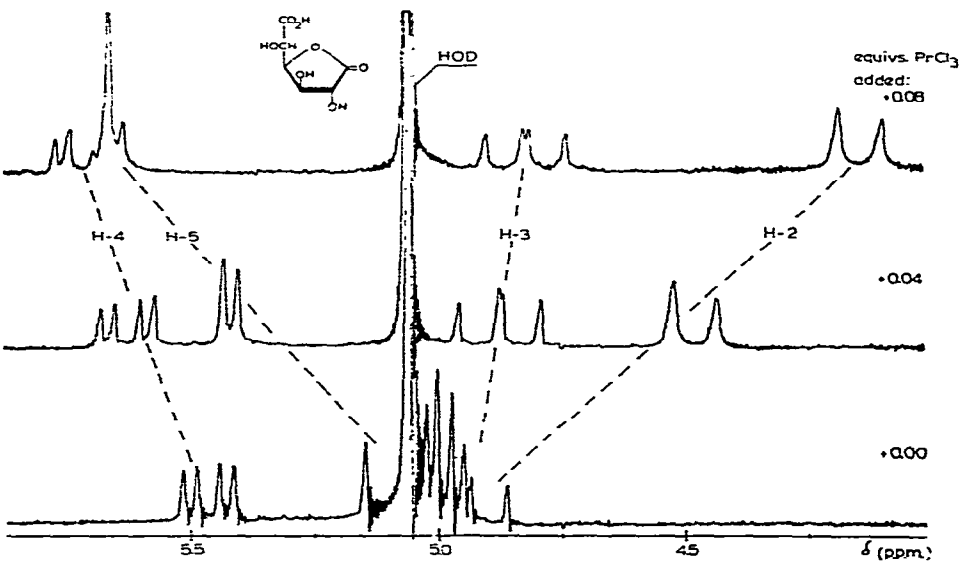


Fig. 2. The 100-MHz, ^{1}H-n.m.r. spectrum of D-glucaro-1,4-lactone (2) in D_2O, without (lower trace), and with (upper traces), the indicated amounts of $PrCl_3$.

TABLE II

100-MHz, ^{1}H-N.M.R.-SPECTRAL DATA FOR D-GLUCARO-1,4-LACTONE (2), MEASURED IN D_2O IN THE PRESENCE OF $PrCl_3$ AT ~30°

Equiv.[a] *of $PrCl_3$ added*	*Chemical shifts (δ values) and lanthanide-induced shifts*[b] *in Δδ (in parentheses)*				*Coupling constants (Hz)*		
	H-2	*H-3*	*H-4*	*H-5*	$^3J_{2,3}$	$^3J_{3,4}$	$^3J_{4,5}$
0.00	5.11d	4.94dd	5.47dd	4.99d	8.9	7.8	2.8
0.04	4.48d	4.87dd	5.63dd	5.42d	8.8	7.8	2.8
	(−0.63)	(−0.07)	(0.16)	(0.43)			
0.05	4.38d	4.86dd	5.67dd	5.50d	8.8	7.8	2.7
	(−0.73)	(−0.08)	(0.20)	(0.51)			
0.06	4.30d	4.85dd	5.70dd	5.57d	8.8	7.8	2.7
	(−0.81)	(−0.09)	(0.23)	(0.58)			
0.07	4.23d	4.84dd	5.71dd	5.62d	8.8	7.6	2.8
	(−0.88)	(−0.10)	(0.24)	(0.63)			
0.08	4.15d	4.83dd	5.72dd	5.65d	8.8	7.5	2.8
	(−0.96)	(−0.11)	(0.25)	(0.66)			
0.12	3.90d	4.79dd	5.78dd	5.82d	8.8	7.7	2.7
	(−1.21)	(−0.15)	(0.31)	(0.83)			
0.16	3.72d	4.76dd	5.83dd	5.96d	8.8	7.8	2.7
	(−1.39)	(−0.18)	(0.36)	(0.97)			
0.20	3.55d	4.74dd	5.89dd	6.08d	8.8	7.8	2.7
	(−1.56)	(−0.20)	(0.42)	(1.09)			
0.24	3.41d	4.73dd	5.94dd	6.19d	8.8	7.8	2.7
	(−1.70)	(−0.21)	(0.47)	(1.20)			

[a]Initial concentration of 2, 63 mg in 0.5 mL of D_2O. [b]Upfield shifts negative.

in D_2O and in Me_2SO-d_6 seem to arise from differences in modes of solvation[25], and they may give information on hydrogen-bonding systems in sugar lactones in solution[30].

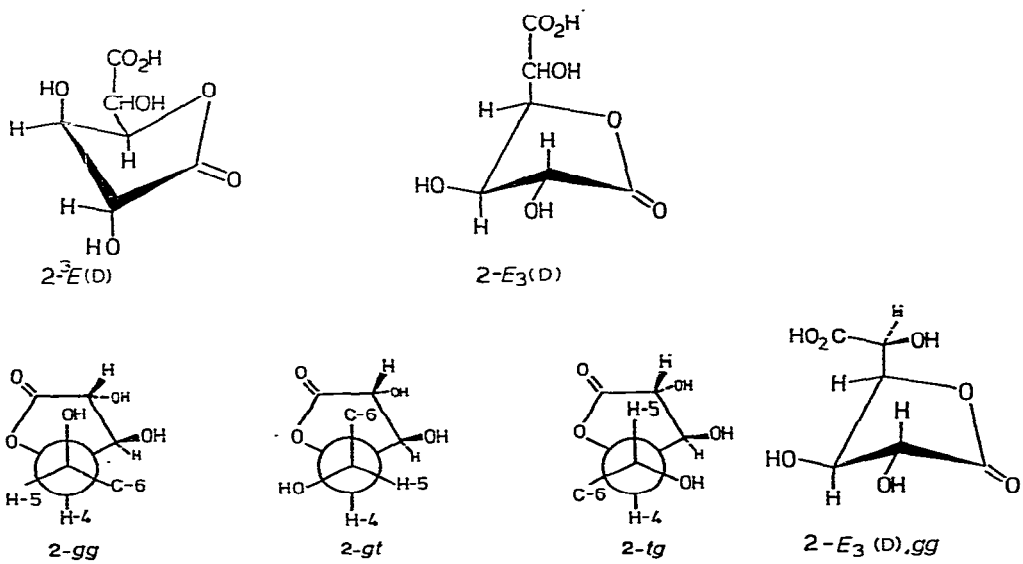
D-*Glucaro-1,4-lactone* (2). — In the ^{1}H-n.m.r. spectra of **2** measured at 100 MHz (see Table I), a doublet of doublets at lowest field (δ 5.47 in D_2O, and δ 5.04 in methanol-d_4) may be confidently assigned to H-4. This proton is strongly deshielded, because of its attachment to the carbon atom adjacent to the partially positive oxygen atom of the lactone ring. This type of deshielding has been observed for a number of non-sugar lactones[20]. The H-4 pattern is well separated from other resonances, and its identification allows assignment of the latter to H-2, H-3, and H-5, although these signals are not very well resolved, either for solutions in D_2O or in methanol-d_4 (see Fig. 1). However, after addition of only very small amounts of the shift reagent praseodymium chloride to a solution of **2** in D_2O, the resonances of H-2, H-3, and H-5 are shifted, either to lower or higher fields, to give well resolved, first-order spectra (see Fig. 2). Vicinal coupling-constants observed in D_2O are $^3J_{2,3}$ 8.9, $^3J_{3,4}$ 7.8, and $^3J_{4,5}$ 2.8 Hz; these values remain practically invariant during addition of $PrCl_3$ (see Table II). The corresponding values for the solution in metha-

TABLE III

CONFORMER POPULATIONS FOR D-GLUCARO-1,4-LACTONE[a] (2) AND D-GLUCARO-6,3-LACTONE (3) IN SOLUTION

Solvent	*Lactone-ring conformation[b] (contributions in percent[c])*		*Exocyclic, $CHOHCO_2H$-group orientation (contributions in percent[d])*	
D-Glucaro-1,4-lactone[c] (2)				
	${}^3E$	E_3	*gg + gt*	*tg*
Deuterium oxide	15	85	85	15
Methanol-d_4	15	85	85	15
D-Glucaro-6,3-lactone (3)				
	E_4	${}^4E$	*gg + tg*	*gt*
Deuterium oxide	~100	~0	50	50
Dimethyl sulfoxide-d_6	~100	~0	35	65

[a]The conformation E_3(D),*gg* was found in the crystal[19]. [b]Symbolism in accord with 1980 IUPAC–IUB Recommendations[31]. [c]Calculated for **2** (from the coupling constants between the cisoidal protons H-3 and H-4) as for D-xylono-1,4-lactone[30], and for **3**, from the coupling constants between the cisoidal protons H-4 and H-5, as for D-lyxono-1,4-lactone[30]. [d]Calculated from the Karplus relation with $J_{60°} = -1.5$ and $J_{180°} = -11.5$ Hz, as for nucleosides[32].



nol-d_4 (8.8, 7.8, and 2.8 Hz, respectively) are very similar to those observed for solutions in D_2O.

The vicinal coupling-constants observed for **2** indicate that, in solution, an equilibrium exists between the conformations discussed next; the conformational equilibrium is described quantitatively by the data given in Table III. The large value

of $^3J_{2,3}$ (8.9 Hz) and also the value of $^3J_{4,5}$ (2.8 Hz) in D_2O strongly indicate that the E_3(D),*gg* conformation illustrated is favored in solution. This conformation was found for **2** in the crystalline state[19]. It should be stressed that similar conformational tendencies were observed for D-xylono- and D-ribono-1,4-lactone[30]. It is possible that a tendency to minimize the total dipole-moment of any particular lactone favors specific conformations, especially those having a quasiequatorial OH-group on C-2. The value of $^3J_{4,5}$ (2.8 Hz) excludes the antiperiplanar orientation of H-4 and H-5, and hence, only their two synclinal orientations can be considered, as far as the E_3(D) conformation is concerned. Placement of the carboxylic group over the lactone ring would lead to large steric interference in a possible alternative to the E_3(D).*gg* conformation.

The relatively large value of $^3J_{3,4}$ (7.8 Hz) in D_2O might result from ring flattening; similar effects have been observed for pyranoid sugars[33], and for five-membered-ring derivatives generally[34-36]. The large value of $^3J_{3,4}$ for **2** might result from synclinal orientation of electronegative substituents at C-3 and C-4, in relation to the coupled protons H-3 and H-4; when not in antiperiplanar relationship to the coupled protons, electronegative groups have been found to increase the magnitude of vicinal, gauche couplings in five-membered rings[37]. When the self-consistent field (SCF), finite-perturbation theory in the intermediate neglect of differential overlap (INDO) molecular approximation was used for calculations of ^{1}H-n.m.r., cisoidal, vicinal coupling-constants in the pentose rings of nucleosides, the theoretical value ($^3J_{3,4}$ = 8.55 Hz) was found for the E_3(D) conformation in *xylo*-nucleosides[38,39].

The fact that both upfield and downfield shifts occur in spectra of **2** measured in the presence of $PrCl_3$ indicates an angular dependence in the dipolar shifts, similar to that observed for sodium (methyl α-D-galactopyranosid)uronate in aqueous solution[40]. Only a very small proportion of $PrCl_3$, namely, 0.04 molar equivalent, was

TABLE IV

CARBON-13 CHEMICAL-SHIFTS FOR **1–5**

Compound	*Chemical shifts in p.p.m. downfield from Me_4Si*[a]					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
1	176.5	74.0[b]	72.2[b]	72.2[b]	72.4[b]	176.25[c]
2	178.05	74.4[b]	72.9[b]	80.7	70.0[b]	178.6
3	174.3	69.7[b]	81.4	71.1[b]	71.3[b]	178.6
4	176.45	71.8	80.95	79.8	69.9	176.45
5	176.45	70.5	77.7	77.7	70.5	176.45

[a]Original data, referenced to the highest-field resonance of DSS, were converted according to the empirical equation[30]: δ (Me_4Si) = δ (DSS) − 1.6. Assignments were made according to general rules applied with 1,4-lactones[21,22,25,26], together with others developed for furanoid sugars[27-29] and various aldonolactones[30]. [b]These assignments may have to be reversed. [c]May be interchanged with the C-1 resonance.

TABLE V

CARBON-13 CHEMICAL-SHIFTS FOR 2–5 IN Me_2SO-d_6

Compound	*Chemical shifts in p.p.m. downfield from Me_4Si, and solvent-induced shift[a] $\Delta\delta$ (in parentheses)*					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
2	175.9 (2.15)	73.7[b] (0.7)	72.3[b] (0.6)	79.3 (1.4)	69.3[b] (0.7)	172.6 (2.45)
3	172.0 (2.3)	69.6[b] (0.1)	80.1 (1.3)	69.8[b] (1.3)	70.5[b] (0.8)	176.2 (2.4)
4	174.0[b] (2.45)	70.4 (1.4)	79.0 (1.95)	78.5 (1.3)	68.3 (1.55)	174.2[b] (2.25)
5	174.15 (2.3)	69.0 (1.5)	75.7 (2.0)	75.7 (2.0)	69.0 (1.5)	174.15 (2.3)

[a] $\Delta\delta$ = δ D_2O − δ Me_2SO-d_6. [b] These assignments may have to be reversed.

necessary in order to provide good separation of signals in the ^{1}H-n.m.r. spectrum of **2**, and addition of $PrCl_3$ at this low concentration caused no appreciable change in the magnitudes of the couplings. These facts indicated[41,42] that the complex of **2** with $PrCl_3$ preserves the geometry of **2**.

Additional support for E_3(D),*gg* as the favored conformation of **2** in solution was afforded by a ^{13}C-n.m.r.-spectral analysis (see Tables IV and V). In D_2O, resonances of C-2 appear at 74.4 p.p.m. downfield from Me_4Si for **2**, and at 71.8 p.p.m. for the dilactone **4** (see Table IV). The relatively large shielding observed for C-2 in **4** seems to be caused by inversion of the quasiequatorial OH group on C-2 in E_3(D)*gg* to the quasiaxial OH group on C-2 in **4** [see **4**-^3E:E_4(D)]; a similar effect was observed for the inversion of an equatorial to an axial oxygen atom in pyranoid sugars[43]. Because the 1,4-lactone ring in the bicyclic molecule **4** is more strained than that in the monocyclic derivative **2**, the C-1 and C-4 resonances are also shifted to higher fields, but not to so great an extent as the resonance of C-2. It is noteworthy that the resonances of C-1, C-2, C-3, and C-4 in **2** appear at fields similar to those of the corresponding resonances[30] in D-xylono-1,4-lactone, which favors the same conformation as **2**.

D-*Glucaro-6,3-lactone* (**3**). — The ^{1}H-n.m.r. spectrum of **3** in D_2O shows only a complex multiplet, at δ 5.05. However, after addition of 0.04 (or more) molar equivalent of $PrCl_3$, manifestly first-order spectra were observed (see Table VI). The spectrum of **3** in Me_2SO-d_6 (see Fig. 3) was not first-order. For **3** in Me_2SO-d_6, a signal observed at δ 4.67 (see Table I) must be assigned to H-2, because only H-2 can adopt an antiperiplanar orientation and give the large coupling-constant observed (8.0 Hz); H-5 in **3** can only be gauche-disposed. Thus, the other resonances observed for solutions in Me_2SO-d_6 (and also in D_2O) may readily be assigned to the individual protons in **3**. The coupling constants given in Tables I and VI indicate that a conformational equilibrium between the ring and side-chain conformations shown exists in solution, and that the ring equilibrium is described quantitatively by the data given in Table III. The values of $^3J_{2,3}$ are slightly smaller than those predicted for the

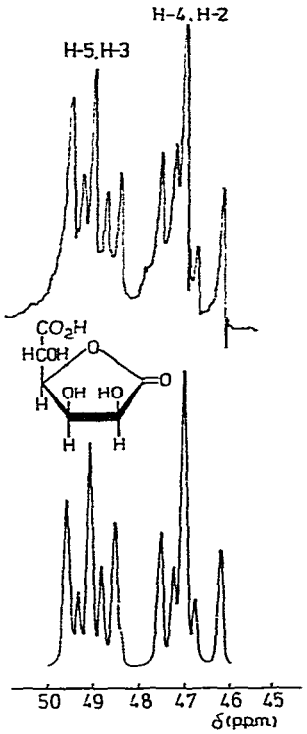
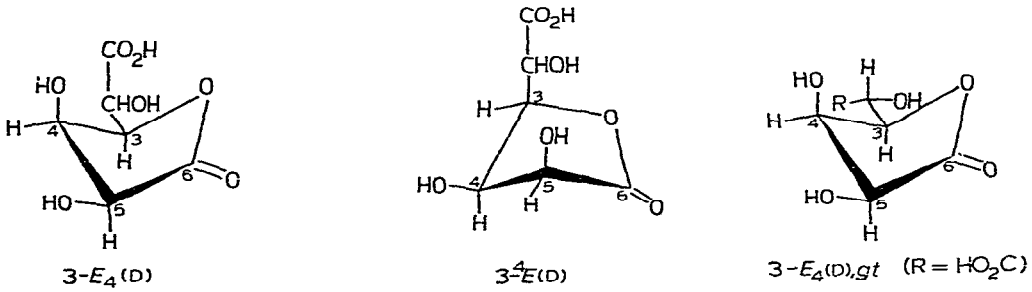


Fig. 3. The 100-MHz, ^{1}H-n.m.r. spectrum of D-glucaro-6,3-lactone (3) in dimethyl sulfoxide-d_6 (upper trace), and the simulated spectrum (lower trace).



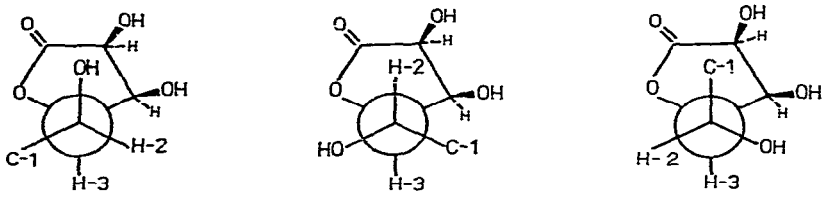


TABLE VI

100-MHz. ^{1}H-N.M.R.-SPECTRAL DATA FOR D-GLUCARO-6,3-LACTONE (3), MEASURED IN D_2O IN THE PRESENCE OF $PrCl_3$ AT ~30°

Equiv.[a] *of $PrCl_3$ added*	*Chemical shifts in δ values, and lanthanide-induced shifts*[b] *in Δδ (in parentheses)*				*Coupling constants (Hz)*		
	H-2	*H-3*	*H-4*	*H-5*	$^3J_{2,3}$	$^3J_{3,4}$	$^3J_{4,5}$
0.00		(multiplet at δ 5.05)[c]					
0.04	5.52d (0.47)	5.32dd (0.27)	5.17dd (0.12)	5.10d (0.05)	6.7	2.5	4.6
0.05	5.60d (0.55)	5.36dd (0.31)	5.19dd (0.14)	5.10d (0.05)	6.7	2.6	4.6
0.06	5.67d (0.62)	5.40dd (0.35)	5.21dd (0.16)	5.11d (0.06)	6.7	2.7	4.7
0.07	5.74d (0.69)	5.43dd (0.38)	5.23dd (0.18)	5.12d (0.07)	6.7	2.7	4.7
0.08	5.82d (0.77)	5.47dd (0.42)	5.26dd (0.21)	5.13d (0.08)	6.8	2.9	4.8
0.12	6.03d (0.98)	5.58dd (0.53)	5.31dd (0.26)	5.14d (0.09)	6.8	2.9	4.8
0.16	6.22d (1.17)	5.67dd (0.62)	5.36dd (0.31)	5.16d (0.11)	6.8	3.0	4.9
0.20	6.38d (1.33)	5.76dd (0.71)	5.40dd (0.35)	5.17d (0.12)	6.8	3.0	4.9
0.24	5.55d (1.50)	5.84dd (0.79)	5.45dd (0.40)	5.18d (0.13)	6.8	3.0	5.0

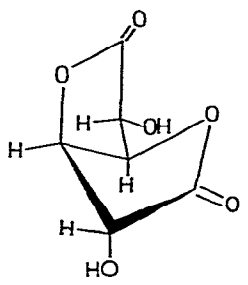
[a]Initial concentration of 3, 57.6 mg in 0.5 mL of D_2O. [b]All shifts downfield. [c]Coupling constants not obtainable by first-order analysis.

antiperiplanar orientation of H-2 and H-3, and consequently, the favored E_4(D),*gt* conformation of 3 may exist in equilibrium with two other rotamers, having H-2 and H-3 synclinally disposed. Very small changes in magnitudes of vicinal couplings during coordination of praseodymium ions (see Table VI) could indicate small changes in the geometry of 3, particularly a slight flattening of the lactone ring, during formation of the complex with $PrCl_3$.

As regards the ^{13}C-n.m.r. spectra, the C-3 and C-5 resonances for 3 (which is less strained than the dilactone 4) appear at fields only slightly lower than the C-3 and C-5 resonances for 4 (see Tables IV and V), suggesting that the E_4(D) conformation of the 6,3-lactone ring, favored for 3, is conserved in 4. The ^{13}C-n.m.r. spectra of D-lyxono-1,4-lactone, D-mannono-1,4-lactone, and D-gulono-1,4-lactone, and, especially, the almost identical positions of the carbon resonances for the lactone ring[30], give additional support for E_4(D) as the favored conformation of the lactone ring in 3, as well as in the aforementioned lactones in solution.

D-*Glucaro-1,4:6,3-dilactone* (4). — Inspection of Dreiding models suggested that the most sterically permissible conformation of 4 should be the ^3E:E_4(D)

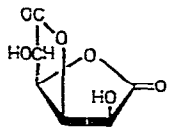
dienvelope. Compound **4** may be considered to be analogous to a *cis*-pentalane derivative, for which a similar, dienvelope conformation was calculated[44] to be the energetically favored form. The ^{1}H-n.m.r. data measured at 100 MHz (see Table I) indicated that the favored conformation of **4** is, indeed, the aforementioned dienvelope. A doublet at δ 4.98 for a solution in D_2O is assigned to the quasiequatorial H-2, which is the most shielded proton in **4** because of its orientation between O-1 and O-3. A small value (0.8 Hz) of $^3J_{2,3}$ in D_2O indicates that H-2 and H-3 are synclinally disposed, with the dihedral angle H-2–C-2–C-3–H-3 close to 90°, in good agreement with the values estimated from Dreiding models. Long-range coupling of 0.4 Hz



4-^3E:E_4(D)

between the quasiequatorial H-2 and the quasiaxial H-4 seems to be similar in magnitude to other long-range couplings across four single bonds between protons having an axial–equatorial orientation[45]. The most deshielded proton is H-4, resonating at δ 5.90 in D_2O, whereas the resonance of H-3 appears at slightly higher field (δ 5.57), because of small shielding by the neighboring group on C-2. There is a synclinal disposition of H-3 and H-4, and also of H-4 and H-5, with values (in D_2O) of $^3J_{3,4}$ 3.9 and $^3J_{4,5}$ 5.4 Hz, respectively. The different magnitudes of the couplings might reflect differences in the orientations of electronegative substituents on C-3, C-4, and C-5. Both of the electronegative substituents on C-3 and C-4 are antiperiplanar to the coupled protons H-3 and H-4, whereas at C-4 and C-5, one of the two electronegative substituents is synclinal, and the other antiperiplanar, to the coupled protons H-4 and H-5. Identical coupling constants were measured[13,14] at 60 MHz during preliminary studies on the conformation of **4**. Earlier ^{1}H-n.m.r. data[46], also measured at 60 MHz in D_2O, are in good agreement with the aforementioned, preliminary data, except for an assignment made by Sawyer and Brannan[46] without according consideration to the correct molecular geometry of **4**.

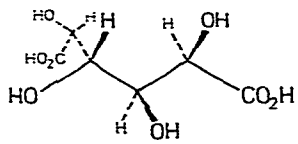
As already pointed out, the ^{13}C-n.m.r. spectra of **3** and **4** suggest conservation, in **4**, of the E_4(D) conformation of the 6,3-lactone ring favored by **3**; on the other hand, the other (1,4) lactone ring in **4** adopts the conformation ^3E(D), having a quasiaxial OH group on C-2. Inversion of the quasiequatorially oriented OH group on C-2 in **2** to the quasiaxial OH group in **4** is accompanied by a relatively large, upfield shift of the C-2 resonance in the ^{13}C-n.m.r. spectrum. Inversion of configuration of



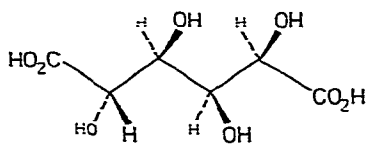
5

C-2 in **4** leads to the increased shielding of C-2, C-3, and C-4 observed for D-mannaro-1,4:6,3-dilactone (**5**), whereas C-5 is deshielded slightly (see Tables IV and V).

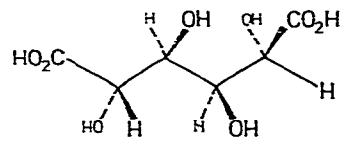
D-*Glucaric acid* (**1**). — In contrast to earlier studies[46], first-order spectra of **1** were obtained at 100 MHz for solutions in deuterium oxide and in methanol-d_4. The chemical shifts and coupling constants given in Table I indicate that equilibrium between various conformations exists in solution. As H-2 is the most deshielded, non-hydroxylic proton, it resonates at lowest field, δ 4.82, in D_2O. The values $^3J_{2,3}$ 2.0, $^3J_{3,4}$ 5.5, and $^3J_{4,5}$ 5.0 Hz suggest[47,48] conformational mixing. The $_3G^+$ form



1-_3G^+



$1\text{-}P$



1-_2G^-

lacks the unfavorable, parallel interaction[47,48] of chain substituents, but it is clearly not the exclusive conformer. The conformational equilibrium of **1** in solution appears to involve the $_3G^+$ and $_2G^-$ sickle forms, together with the planar (*P*), zigzag conformation (for an explanation of the symbolism, see refs. 47 and 48). It may be noted that the $_3G^+$ and *P* conformations have been found for D-gluconate ions in the crystalline state[49,50].

The ^{13}C-n.m.r. spectra also suggest conformational similarity between **1** and D-gluconic acid; the resonances of C-1, C-3, and C-4 in the ^{13}C-n.m.r. spectrum of **1** (see Table IV) appear at fields almost identical to those of the corresponding resonances in the ^{13}C-n.m.r. spectrum of D-gluconic acid; the C-2 nucleus in **1** is slightly deshielded[30]. The $_2G^-$ and $_3G^+$ conformations were respectively found[51] for the crystalline calcium and potassium salt of **1**, and the same conformations are here suggested for the compounds in solution.

^{13}C-N.m.r. spectroscopy was found to be convenient for monitoring the lactonization of **1**, and the hydrolysis of its lactones. It was clearly demonstrated (data not shown) that, at 30°, **1** lactonizes spontaneously to give **3** as the first product, but that compound **2** forms more slowly. No lactones other than **2**–**4** were found in solutions prepared from **1**–**4** (see Table VII), either during mutarotation or after equilibration. Thus, the hypothetical D-glucaro-1,5-lactone[52] was not formed in a detectable amount under the conditions described here.

TABLE VII

EQUILIBRIUM COMPOSITION OF SOLUTIONS OF D-GLUCARIC ACID (1) AND ITS LACTONES

Solvent	*Content of the acid and lactones (in percent)*		
	Acid (1)	*1,4-Lactone* (2)	*6,3-Lactone* (3)
A[a]	40	30	30
B[b]	40	30	30

[a]Solution A, ~27%, in D_2O at ~30° (this work); the composition calculated from the total peak-areas of protonated carbon atoms in the ^{13}C-n.m.r. spectra, according to the procedure described elsewhere[30]. [b]Solution B, ~10%, in water at ~30°; composition determined by quantitative, paper chromatography[54].

EXPERIMENTAL

Materials. — D-Glucaro-1,4-lactone (2) monohydrate, D-glucaro-6,3-lactone (3), D-glucaro-1,4:6,3-dilactone (4), and D-mannaro-1,4:6,3-dilactone (5) were obtained by methods described earlier[13,14]. D-Glucaric acid (1) was prepared according to a slight modification of Rehorst's method[53]. Compounds 1–5 had physical constants in good agreement with published values. Chromatographic homogeneity of the lactones and of the acid was proved by paper chromatography according to the method described by Hirasaka *et al.*[54], and by thin-layer chromatography (t.l.c.) on Silica Gel G (E. Merck, Darmstadt, GFR).

^1H-N.m.r. spectra. — Spectra were recorded at 100 MHz with a Varian HA-100 n.m.r. spectrometer in the frequency-sweep mode at a probe temperature of ~30°. After exchange of the hydroxyl-group protons by dissolution in D_2O, solutions of the compounds in D_2O, methanol-d_4, or dimethyl sulfoxide-d_6 were prepared by dissolving each compound (0.2–0.3 mmol) in the solvent (0.4–0.6 mL). Solutions also contained 5% (w/v) of sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) (or, in some cases, acetonitrile or acetone) or tetramethylsilane, as the internal standard and to provide a lock signal. A solution of $PrCl_3$ in D_2O containing 3 μmol of $PrCl_3$ per drop (~4 μL) was added dropwise, with continual, spectral scanning to monitor the effect of each addition. The solution was prepared from $PrCl_3 \cdot 6\ H_2O$ (Alfa-Ventron). The hexahydrate was dried at ~78° over P_2O_5 *in vacuo* during 4 h, in order to remove practically all of the water. The residue was dissolved in D_2O (2 vol.), the solution lyophilized, and the residue redissolved in D_2O. Computer-simulated spectra were generated, with the aid of the program LAOCOON III, in order to verify the measured ^1H-n.m.r. chemical-shifts and coupling constants.

^{13}C-N.m.r. spectra. — Proton-decoupled, natural abundance-carbon-13, pulse Fourier-transform (p.F.t.) n.m.r. spectra were recorded with a Bruker HX-90 multinuclear spectrometer, in part by Dr. C. Cottrell of The Ohio State University. The compound (0.4 g) was dissolved in 1.5 mL of D_2O or Me_2SO-d_6, and DSS or

Me_4Si was added as the internal standard. Spectra were recorded at ~30° by using the deuterium resonance of D_2O or Me_2SO-d_6 as the lock signal, and they were referenced either directly to the internal Me_4Si or to the highest-field resonance of DSS, and then converted according to the equation given in Table IV. By using internal 1,4-dioxane as an additional internal reference, it was found that the highest-field resonance of DSS is upfield by 1.6 p.p.m. with respect to Me_4Si. Measurements were made at 22.63 MHz, with proton broad-band decoupling at 90 MHz. Typical measurement conditions were as follows: number of scans 1000–4000, data points 8K or 16K, repetition frequency 0.8 or 1.6 s, sweep-width 5 KHz. The spectra of each compound in D_2O were recorded immediately after dissolution, during mutarotation, and when mutarotation was complete.

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