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EQUILIBRIA OF THE ANOMERIC AND RING FORMS OF AMMONIUM 3-DEOXY-D-*manno*-OCTULOSONATE IN DEUTERIUM OXIDE

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ABSTRACT

The tautomeric composition of a solution of ammonium 3-deoxy-D-*manno*-octulosonate (KDO, **1a**) in D_2O at 28° was assessed by means of ^{13}C-F.t.-n.m.r. spectroscopy. The results revealed the presence of ~60 and 11% of the α and β anomers of the pyranose, and 20 and 9% of the two furanoses, and suggested, but did not unequivocally prove, that the major furanose form is the α anomer. To facilitate interpretation of the spectral results for **1**, ammonium 3,5-dideoxy-D-*arabino*(or *ribo*)-octulosonate (**3a**) was prepared by the reaction of 2-deoxy-D-*erythro*-pentose with sodium oxalacetate at pH 11. A chromatographically homogeneous, noncrystalline sample of **3** was obtained by lyophilization, and characterized as its (4-nitrophenyl)hydrazone (m.p. 162–163°). The ^{13}C-n.m.r. spectrum of a solution of **3a** in D_2O revealed it to be substantially all in the α-pyranose form. No signals were obtained for the possible 1,4-lactone of **3**. As the 1,5-lactone and furanose forms are impossible for **3**, it exhibited no signals analogous to those attributed to furanoid **1**. On the basis of these results for **3**, the two lactone forms of **1** were excluded from consideration, and the three pairs of ^{13}C-n.m.r. signals observed at ~45, 86, and 104 p.p.m. were assigned to the furanose forms of **1**.

INTRODUCTION

The cell envelope of most Gram-negative bacteria contains a highly immunogenic, complex lipopolysaccharide that is the principal constituent of the bacterial endotoxin. The central component of this complex structure, namely, 3-deoxy-D-*manno*-octulosonic acid (KDO, **1**) provides the linkage between the complex, polysaccharide antigen and lipid A. The synthesis of the inner portion of the lipopolysaccharide, containing the complete KDO–lipid A structure, is essential for growth. Therefore, any disruption in the synthesis, activation, or transfer of **1** severely affects the organism.

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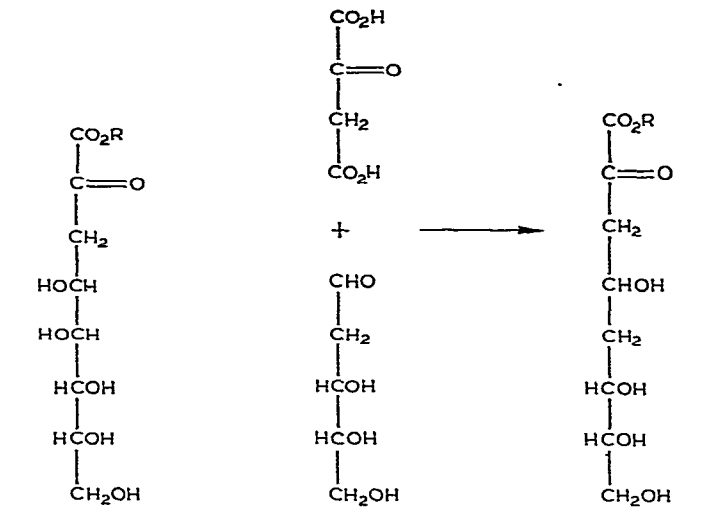


As a prelude to the study of the effect that analogs of **1** have on the metabolism of **1**, we synthesized the ammonium salts (**1a** and **3a**) of **1** and of an analogous 3,5-dideoxyoctulosonic acid (**3**). The availability of these compounds made possible the study, by ^{13}C-n.m.r. spectroscopy, of their anomeric and ring-form equilibria in solution in deuterium oxide.

The application of ^{13}C-n.m.r. spectroscopy to the analysis of carbohydrate solutions in order to determine the percentages of the anomeric furanose and pyranose forms present is now well established[1-3], particularly for ketoses. Complications from differences in relaxation rates and in magnitudes of the nuclear Overhauser effect (n.O.e.) for otherwise comparable signals can, in principle, lead to errors in interpretation of integrated, pulsed, Fourier-transform (F.t.), ^{13}C-n.m.r. spectra. However, reasonably accurate results should be obtainable if care is taken in choosing the experimental conditions[4], and if the signals integrated are for carbon atoms having very similar attachments, such as the anomeric carbon atom in each species of concern. Certainly, in assessing the composition of solutions, ^{13}C-n.m.r. spectra are of outstanding value where other techniques, such as ^{1}H-n.m.r. spectroscopy, are inapplicable.

The ^{1}H-n.m.r. signal from the hydrogen atom bound to the anomeric carbon atom has proved very useful for determination of the tautomeric equilibria of aldoses. The necessary signals are frequently well resolved from each other, and far removed from other signals in the spectra of typical aldoses[5,6]. As ketoses possess no anomeric hydrogen atom, they are not amenable to analysis by ^{1}H-n.m.r. spectroscopy, but are readily studied by ^{13}C-n.m.r., spectroscopy. The clear applicability of the technique to **1**, the absence of any report of evidence for the existence of furanose forms of this

important carbohydrate, and the appearance of a recent article[7] attributing to lactone forms certain ^{13}C-n.m.r. signals of low intensity for a solution of the sodium salt (**1b**) of **1** prompt the present communication. The purposes of this work were to (*a*) corroborate assignments of the ^{13}C-n.m.r. chemical shifts[7] for a pyranose form of **1a** strongly preponderating over other isomeric forms in solution in deuterium oxide; (*b*) report, for the first time, chemical shifts of other ^{13}C signals, of lower intensity, which we attribute to the anomeric pyranose and the anomers of the furanose form; (*c*) assess the composition of the equilibrated solution quantitatively in terms of the four tautomers observed; and (*d*) report the preparation and purification of **3a**, and the ^{13}C-n.m.r.-spectral results therefor, which were used comparatively as indirect evidence for the presence of furanose, rather than lactone, forms of **1a** in aqueous solution.

EXPERIMENTAL

Analytical methods. — Pentose was determined by the phenol–sulfuric acid method of Smith *et al.*[8]. The deoxyoctulosonic acids were estimated by the thiobarbituric acid method of Weissbach and Hurwitz[9], as modified by Osborn[10], and by the semicarbazide method of MacGee and Doudoroff[11].

Paper chromatography was conducted by the ascending technique, using Whatman No. 1 paper with solvent system *I*: 5:5:3:1 (v/v) ethyl acetate–pyridine–water–acetic acid. Thin-layer chromatography was performed with Silica Gel G with the following solvent systems: *II*, 11:9 (v/v) benzene–acetone; *III*, 5:5:1:2 (v/v) ethanol–pyridine–water–acetic acid; and *IV*, 5:3:2 (v/v) 1-butanol–pyridine–0.1M hydrochloric acid. High-voltage, paper electrophoresis was conducted on Whatman No. 1 paper at 6 kV for 0.5 h in a buffer system [1:10:69 (v/v) pyridine–acetic acid–water, pH 3.6]. Reducing sugars were detected with alkaline silver nitrate, and the deoxyoctulosonic acids by the thiobarbituric acid method of Warren[12]. All carbohydrate starting-materials were obtained from Sigma Chemical Company.

N.m.r. spectra. — The ^{13}C-n.m.r. spectra were recorded by use of either a JEOL JNM/FX-60 or a JNM/FX60Q n.m.r. spectrometer operating at 15.04 MHz and equipped with a 10-mm, $^{1}H/^{13}C$ dual probe, a Texas Instruments Model 980B computer with 24K Memory, and a JEOL Model NM 5471 temperature-controller. Unless otherwise noted, the spectra were recorded at 28° by using the pulsed-fast, Fourier-transform method, employing the deuterium resonance of the D_2O solvent for internal lock, with 4-kHz spectral-width, collection of 8K data points in the f.i.d., proton decoupling by a 1-kHz noise-band centered at 48.00-kHz offset-frequency, and with a 45° pulse repeated at intervals of 15 s. The chemical shifts were determined relative to tetramethylsilane (Me_4Si) through the use of an internal, 1,4-dioxane reference taken at 67.40 p.p.m. relative to Me_4Si. All spectra used for integration of signal areas were determined by using gated decoupling techniques which provided complete decoupling without n.O.e. Carbon–hydrogen coupling-constants were obtained from spectra determined with no decoupling.

Preparation of **1a**. — Ammonium 3-deoxy-D-*manno*-octulosonic acid (**1a**) was prepared by the method of Ghalambor *et al.*[13], as modified by Hershberger *et al.*[14], but was purified by the following modification. The reaction mixture was made neutral, and applied to a column (6.5 × 10 cm) of Dowex-1 X-8 (HCO_3^-) ion-exchange resin (200–400 mesh). The column was washed with water until the eluate gave a negative test for arabinose by the phenol–sulfuric acid procedure. Compound **1a** was then eluted with 0.5M ammonium hydrogencarbonate at a flow rate of 60 $mL.h^{-1}$, and 15-mL fractions were collected. Alternate fractions were analyzed by ascending paper-chromatography in solvent *I*, or by high-voltage electrophoresis. Appropriate fractions were pooled, and the ammonium hydrogencarbonate was removed by repeated lyophilization, or evaporation below 40°. Compound **1a** was dissolved in hot, 85% ethanol (1.3 g per 40 mL) and, after several min at room temperature, the solution was nucleated, and placed in a refrigerator. The first crop of crystals of the ammonium salt of **1** weighed 1 g. The physical and chromatographic properties of the product were consistent with those reported[14]. The sample of this salt employed for ^{13}C-n.m.r. studies had been twice recrystallized.

The elution with 0.5M ammonium hydrogencarbonate gave three pooled fractions as determined chromatographically. The material in fractions 11–30 was a single species that corresponded chromatographically to authentic ammonium salt (**1a**) of **1**. Fractions 31–46 contained this ammonium salt and that of the 3-carboxy derivative of **1**, with the latter preponderating. Fractions 47–61 contained traces of the ammonium salts of **1**, of the 3-carboxy derivative, and of an unidentified product. Crystalline **1a** was obtained directly from the first two pooled fractions on removal of the ammonium hydrogencarbonate.

Preparation of **3a**. — Ammonium 3,5-dideoxy-D-*arabino*(or *ribo*)-octulosonic acid (**3a**) was prepared as for **1a**, except that 2-deoxy-D-*erythro*-pentose was substituted for D-arabinose. The **3a** obtained by ion-exchange chromatography and lyophilization was purified by partition chromatography on a column (2.5 × 35 cm) of Sephadex LH-20 equilibrated with 17:3 acetone–water, as attempts to crystallize it were unsuccessful. The column was eluted with 17:3 acetone–water at a flow rate of 75 $mL.h^{-1}$, and 15-mL fractions were collected. Every other fraction was assayed by the thiobarbituric acid procedure, and by paper chromatography in solvent *I*. Fractions 43–89 contained only **3a**, exhibiting a relative mobility of 1.2 compared to that of **1a**, and were combined as follows: 43–53, 54–73, and 81–89, to give pooled fractions I, II, and III, respectively. Later fractions were discarded. The pooled fractions were evaporated *in vacuo* below 40°, triturated with ether, dissolved in water, and the solution lyophilized. Pooled fraction I was used for the ^{13}C-n.m.r. studies, and for elemental analysis.

Anal. Calc. for $C_8H_{17}NO_7$: C, 40.17; H, 7.11; N, 5.86. Found: C, 40.15; H, 7.34; N, 5.51.

Compound **3a** (100 mg) was treated with (4-nitrophenyl)hydrazine (100 mg) in 17:3 ethanol–water. The mixture was heated on a boiling-water bath for 5 min, or until all of the material had dissolved, and the mixture was kept overnight in a

TABLE I

CARBON-13 CHEMICAL SHIFTS[a] AND C–H COUPLING-CONSTANTS[b] FOR 0.18M SOLUTIONS IN D_2O AT 28°

Carbon number	*Pyranoses*			*Furanoses*[c]	
	α-**3a**[d]	α-**1a** *major*	β-**1a** *minor*	**1a** *minor*	**1a** *major*
1	177.7s	177.7s	176.3s[e]	177.8[e]	178.4s[e]
2	97.7s	97.2s	98.2s	103.8s	105.0s
3	40.8t (132)	34.5t (131)	35.9t	44.5t (133)	45.5t (133)
4	64.8d (140)	67.0d (143) or 67.4 (144)			73.3d (153)
5	34.5t (127)	67.0d (143) or 67.4 (144)		85.8d (148)	86.4d (148)
6	71.1d (142)	72.0d (142)			71.7 or 72.3
7	74.2d (145)	70.0d (145)			72.3 or 71.7
8	62.9t (143)	63.8t (143)			
Unassigned signals			64.6, 66.3, 68.4, 70.5, 71.5, 74.4		

[a]Measured in p.p.m. relative to internal 1,4-dioxane taken as 67.40 p.p.m. relative to Me_4Si. [b]Values in Hz, given in parentheses. Signals designated d, s, and t for doublet, singlet, and triplet. [c]Furanose C-4,6,7,8 signals are tentatively assigned. [d]Signals for the minor component (presumably the β-pyranose) were apparent, but might be due to unseparated impurities; thus, the chemical shifts are not included here. [e]At 200 mg/1.00 mL.

refrigerator. The (4-nitrophenyl)hydrazone that crystallized out was collected by filtration, and recrystallized twice from 17:3 ethanol–water. Thin-layer chromatography (t.l.c.) in solvents *II* and *III* indicated a single, major component and a trace of (4-nitrophenyl)hydrazine. The derivative was purified by preparative t.l.c. The material corresponding to the (4-nitrophenyl)hydrazone of the ammonium salt of **3** was eluted with methanol, and recrystallized from 17:3 ethanol–water; m.p. 162–163°.

Anal. Calc. for $C_{14}H_{22}N_4O_8$: C, 44.92; H, 5.88; N, 14.97. Found: C, 45.48; H, 5.61; N, 15.16.

RESULTS

The ^1H-decoupled, ^{13}C-n.m.r. spectrum of an isomerically equilibrated solution of **3a** in D_2O consists of a set of eight prominent resonances and a set of minor ones having amplitudes only ~3–5% of those of the former. For the prominent resonances, off-resonance-decoupled and ^1H-coupled spectra provided signal multiplicities and carbon–hydrogen coupling-constants as given in Table I, column 2, along with the chemical shifts observed. The intensity of the signal at 40.8 p.p.m. was greatly di-

TABLE II

PERCENT COMPOSITION[a] OF 0.18M AND 0.72M SOLUTIONS OF **1a** IN D_2O AT 28°

^{13}C-Signal (Integrated)	α-Pyranose		β-Pyranose		Furanose-I		Furanose-II	
	0.18M	0.72M	0.18M	0.72M	0.18M	0.72M	0.18M	0.72M
C-1	—	64	—	6	—	20	—	10
C-2	61	64	11	5	19	20	9	11
C-2[b]	—	68	—	5	—	19	—	8
C-3	59	62	11	6	21	20	9	11
C-5	—	—	—	–	20[c]	22[c]	10[c]	9[c]
Average	60	64	11	6	20	20	9	10

[a]Determined with a spectral width of 4 kHz with 8K data points in the f.i.d., except as noted in footnote b. [b]Sample aged for 6 weeks, and determined at a spectral width of 200 Hz with 8K data points in the f.i.d. [c]Assuming that the furanoses constitute 30% of the total mixture.

minished, and small, symmetrical, satellite signals appeared near its base when the decoupled spectrum was repeated on a sample from which D_2O had been lyophilized three times. This effect was enhanced by aging of the final D_2O solution. No other significant spectral-changes were observed for the aged sample.

The ^{13}C-n.m.r. spectrum of an isomerically equilibrated, D_2O solution of **1a**, obtained by using gated ^1H-decoupling to avoid n.O.e., consisted of three groups of signals which differed in relative, integrated intensities. The most intense set, of eight signals (see Table I, column 3), appeared over the entire, spectral range from 34–178 p.p.m. Other spectra for the same sample revealed the multiplicities, and provided carbon–hydrogen coupling-constants for these signals. The secondmost intense set of signals (Table I, column 6) had amplitudes about one third those of the first. Of these, only seven signals could be clearly identified, but another is probably coincident with one of the signals (at 63.8, 67, or 67.4 p.p.m.) of the former group. The third group of signals (Table I, columns 4 and 5) were of very low intensity, generally ~30–50% as intense as the signals of the second group. In this set, only thirteen signals could be clearly distinguished. Certain signals from this third group seemed clearly associated with particular absorptions in the first two groups when the latter were well separated from other signals. Whenever this occurred, the multiplicities of the lower-level signals corresponded with those of the associated, stronger absorptions. The relative, integrated intensities of selected signals in these various sets are given in Table II.

DISCUSSION

The ^{13}C-n.m.r. spectra and chemical-shift assignments of the α-pyranose form of **1b** and the analogous methyl α- and β-glycosides have recently been reported[7]; none of the signals of the β-pyranose form of **1b** were detected, but discernible signals

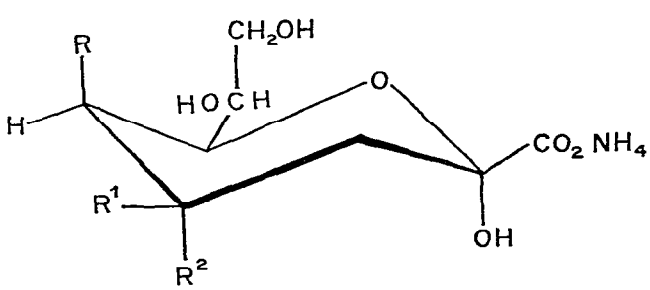
at 86.8, 86.3, 45.8, and 44.7 p.p.m. were attributed to C-4, C-5, and C-3 of the five- and six-membered rings of the two lactones of **1**.

The results reported herein include the observation of low-intensity signals attributable to the β-pyranose form of **1a**. As **3** has a deoxy center at C-5, it can form neither the (less stable) 1,5-lactone nor the anomers of the furanose form, but it could, theoretically, form the (more stable) 1,4-lactone involving O-4. The absence from the spectrum of **3a** of all of the signals earlier attributed to the two lactone forms of **1** is compelling evidence against the presence of either of the lactones and for the presence of the furanose forms of **1** in its basic, aqueous solutions. Furthermore, in the chemical-shift range of the anomeric and deoxy carbon atoms, the spectrum reported herein for **1a** is extremely similar in relative chemical-shifts and intensities to that published[15] for 2-deoxy-D-*erythro*-pentose, which is a partial structural analog of **1**, and which is recognized as existing in all four of its pyranose and furanose forms[15].

As **3** lacks the 5-hydroxyl group necessary for formation of furanose or 1,5-lactone forms, its eight prominent, ^{13}C signals are undoubtedly due to a single pyranose form that strongly dominates the anomeric equilibrium. At this time, it cannot be unequivocally demonstrated that the configuration of C-4 in **3** (the chiral center formed during the preparation of the octulosonic acids described herein) is the same as that established[16] for **1**; but the n.m.r. data for **3a** suggest that it is. However, for the purposes of the following discussion, the configuration of C-4 is assumed to be unimportant. Therefore, it would be anticipated that the presence of the $^5C_2$(D) conformation of the α anomer (**4**) of **3a** would be almost exclusively responsible for the eight prominent signals observed, just as it is for those of the α anomer (**5**) of **1a**.

This conformation has an equatorial disposition for the C-7,C-8 grouping attached to C-6; this would control the conformational equilibrium, for the 4-hydroxyl group having the same attachment as OH-4 in **1**, and for the carboxylate group on C-2, a disposition strongly favored over that disposition for the 2-hydroxyl group by analogy to conformational equilibrium-constants for substituted cyclohexanes[17] and the anomeric effect[6] of a 2-hydroxyl group adjacent to a deoxy carbon atom (C-3).

Columns 2 and 3 of Table I give the chemical shifts for the preponderant forms



4 R = H, R^1 = OH or H, R^2 = H or OH

5 R = OH, R^1 = OH, R^2 = H

of **3a** and **1a**. The most downfield signal, at ~178 p.p.m. for each of these two pyranoses, which is in the spectral region where carbonyl groups are known to absorb, is assignable to the carboxylate carbon atom (C-1). The other singlets (97.7 and 97.2 p.p.m.) for these species are due to the C-2 atoms, as these can exhibit no direct C–H coupling, and as anomeric carbon atoms generally absorb somewhat downfield from singly oxygenated, carboxylate carbon atoms[18].

The most downfield triplets (62.9 and 63.8 p.p.m.) are due to the C-8 atom, as the observed multiplicity and chemical shifts are characteristic of hydroxymethyl groups of carbohydrates[1–3,18].

The remaining triplets in the spectrum of **3a** are less readily assignable. The preponderating, pyranose form (**5**) of **1** differs from **4** by replacement of a hydrogen atom at C-5 in **3** by an axial hydroxyl group (in **5**). An identical replacement at C-4 in *tert*-butylcyclohexane produced[19] downfield shifts of its ^{13}C resonances for C-4 (α, 37.8 p.p.m.) and C-3,5 (β, 5.5 p.p.m.), and a significant upfield shift (γ, 6.8 p.p.m.) of the resonances for its γ-carbon atoms, C-2 and 6. In remarkable agreement with these data, the triplet at 40.8 p.p.m. for **3a** is assigned to C-3, which is gamma to C-5, and exhibits an upfield shift of 6.3 p.p.m. to the δ value of 34.5 p.p.m. for **1a**. The similarity in the C–H coupling-constants for these two signals serves as corroborating evidence for their assignments, as does disappearance of the signal in an aged, D_2O solution of **3a**. This disappearance is undoubtedly produced by deuterium exchange, which should be facile at this carbon atom (*alpha* to the anomeric center).

The foregoing ^{13}C data for the cyclohexane derivatives may be applied generally for correlating, but not strictly for calculating, the relationship between the remaining signals in columns 2 and 3 of Table I. The relationships calculated are not likely to be accurate, as it is commonly known that substituent effects are non-additive in the spectra of inositols and carbohydrates. The calculated chemical shifts relative to Me_4Si are, in most instances, larger than those actually observed[20]. The resonance of C-5 in **1a** would be shifted downfield from the C-5 resonance for **3a** (34.5 t) to

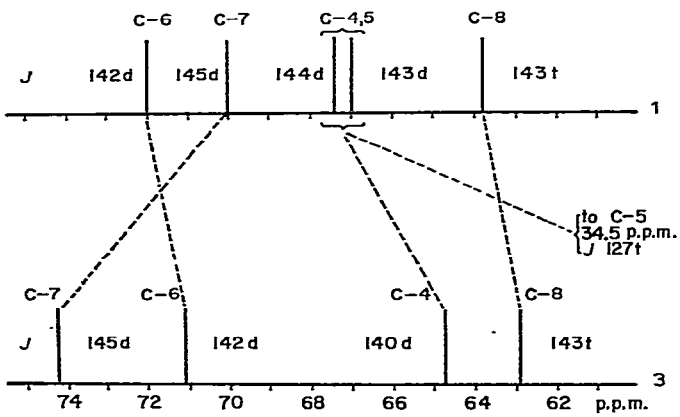


Fig. 1. Correlation of selected, carbon-13 chemical-shifts of the ammonium salts of the α anomers of the pyranose forms of **1** and **3**, as 0.18M solutions in D_2O at 28°.

~72 p.p.m. (34.5 + 37.8) due to the *alpha*-substituent effect of its axial O-5 atom. This value is probably larger by a few p.p.m. than would actually be observed, and any of the five doublets observed between 63 and 72 p.p.m. for **1a** could be due to C-5. As C-4 and C-6 in **1a** are *beta* to the axial, O-5 atom, they should be shifted downfield only slightly (<5.5 p.p.m.) from the corresponding signals for **3a**, while C-7 and C-3, being *gamma* to O-5, should be shifted somewhat upfield (<6.8 p.p.m.). These relationships may be reconciled by the correlation diagram given in Fig. 1, where the more-downfield doublet for the C-4, C-6 shieldings of **3a** has been attributed to C-6, as it is attached to the oxygen atom of the pyranose ring and is *beta* to the hydroxylated C-7 atom. The close spacing of the C-4,C-5 resonances in the spectrum of **1a** causes their assignment to these individual carbon atoms to be uncertain, and this has been noted in Table I.

The C-2 shieldings for the two α-pyranoses are similar, and afford at least minor support for the assumption that **3** is an *arabino*- rather than a *ribo*-octulosonic acid and possesses the same configuration of C-4 as does **1**. The δ-substituent shift reported for the cyclohexane derivatives is 0.7 p.p.m. (upfield for axial OH substitution)[19]. The C-2 shielding observed for **1a** is 0.5 p.p.m. upfield of that for **3a**. If the configuration of C-4 (γ to C-2) were reversed in **3**, the two C-2 resonances would probably not be so similar. Indeed, were the configuration reversed in the sample of **3a** examined, the C-2 shielding for **4** could be computed from the observed value (97.7 p.p.m.) and the Dorman and Roberts[21] γ_x parameter (2.8 p.p.m. upfield) for inversion of C-4 (axial OH to equatorial OH). The resonance thus corrected would occur at ~94.9 p.p.m. Addition of a δ-hydroxyl group at C-5 would lead to the prediction of a C-2 resonance for **1a** still farther upfield, well above the value (97.2 p.p.m.) actually observed.

The ^{13}C resonances of carbon atoms of furanoses are generally recognized to absorb substantially downfield of those of the configurationally related pyranoses[3]. On the basis of this observation, as well as their observed, relative intensities and their order of multiplicity in ^1H-coupled spectra, the signals for C-1 through C-3 were assigned to the furanose forms of **1a**, as indicated in columns 5 and 6 of Table I. The resonances (doublets in ^1H-coupled spectra) of comparably low intensity at ~86 p.p.m. were assigned to C-5 of the furanoses, on the basis of their large deshielding[20,22] produced by the *O*-alkylation in formation of the furanose ring. The other assignments for the major, furanoid component are tentative.

Column 4 of Table I gives the assignments of signals for C-1 through C-3 of the β-pyranose form of **1a**, based on the close relationship between these signals and those of the α-pyranose.

Included separately in Table I are six resonances of low intensity which were not clearly associated with individual resonances of major components in the solution. The assignment of several of these to the β-pyranose could be made by analogy to the resonances published for the methyl α- and β-pyranosides[7]. Due to the narrow range of their chemical shifts, however, and their close similarity to several signals of the major components, their assignments would be very uncertain.

Table II shows that, in D_2O solution, ~30% of **1a** exists as a 2:1 ratio of the anomeric furanoses. The anomeric configuration of the preponderant furanose form is suggested by, but cannot be unequivocally determined from, the relative chemical-shifts given herein.

Except for the carboxylate group (C-1) and the greater length of the side chain (C-6 through C-8), the β-furanose form of **1** is a structural analog of the α-furanose form of 2-deoxy-D-*erythro*-pentose (**2**). Breitmaier *et al.* showed[15] that the anomeric carbon atom is more shielded in the α- than in the β-furanose form of **2**. For **1a**, the anomeric carbon atom of the minor furanose is more shielded than that of the major furanose. Therefore, the major furanose probably has the α-anomeric configuration. Although, the structural imperfections of this analogy open the conclusion to question, it is strengthened by the fact that the relative chemical-shifts of the anomeric carbon atoms in the methyl α- and β-furanosides of neither D-psicose nor D-fructose[23] (having C-1 and C-6 relationships similar to the C-1 and side-chain relationship of **1**) change in relationship to those of the analogous methyl D-ribofuranoside and D-arabino-furanoside, respectively[22], which lack C-1, C-6 interactions.

The C-5 chemical-shifts of the furanose forms of **1a** offer evidence that apparently contradicts the foregoing conclusion regarding an excess of the α- over the β-furanose. The major furanose form of **1a** exhibits a less-shielded C-5 atom than does the minor furanose form; this suggests that the major furanose form has the β-anomeric configuration, in which O-2 and O-4 are *cis*-disposed. Generally, for methyl aldo-pento- and -hexo-furanosides[22,23] and 2-hexulofuranoses[24,25], it has been observed that relationships similar to this cause C-4 to be less shielded when O-3 and O-1 are cis in the aldofuranosides, and C-5 to be less shielded when O-4 and O-2 are cis in the 2-ketofuranoses. This generalization has apparently not, however, been investigated for monosaccharides having a deoxy center adjacent to the anomeric center. It is interesting that the partial structural analog to **1** mentioned earlier, namely, **2**, exhibits less shielding for C-4 of its major furanose form[15], just as does **1a** for its C-5 atom, and in contrast to the ribofuranoses[15,26].

Table II shows that the anomeric equilibrium of the pyranose forms is dominated by the α anomer in the ratio of ~10:1 over the β anomer. There is probably a strong tendency for each of these anomers to adopt the $^5C_2$(D) conformation. If this is so, or if the relative thermodynamic stabilities of the anomers are differentially unaffected by contributions from the $^2C_5$(D) conformations, reasonable values for all of the net, group interaction-energies affecting the equilibrium are available, except that for the anomeric effect of the carboxylate group itself. Therefore, the data in Table II may serve as the basis for estimating the latter quantity. Such an estimate provided a value of ~1.9 kJ.mol^{-1} for the anomeric effect of the anionic carboxylate group.

APPENDIX

Estimation of the anomeric effect of the anionic carboxylate moiety, in 0.72M

NH_4 salt of **1** *dissolved in* D_2O. — The group interactions are: for $^5C_2(D)_\alpha$ (X) (*a*) gauche O-5: C-7; (*b*) gauche O-4: O-5; (*c*) three O_a: H_a; and (*d*) the anomeric effect of CO_2^-; and for $^5C_2(D)_\beta$ (Y) (*i*) gauche O-5: C-7; (*ii*) gauche O-4: O-5; (*iii*) one O_a: H_a; (*iv*) two CO_2^-: H_a; and (*v*) the anomeric effect of OH.

Assuming no differential contributions from the $^2C_5(D)$ conformations, the net free-energy difference from $\Delta G^0 = G^\circ_Y - G^\circ_X$ is:

$$\Delta G^0 = (iii)\text{-}(c) + (iv) + (v) - (d)$$
$$= -2 \times 1.9 \text{ (from ref. 17, p. 356)} + 2 \times 4.0 \text{ (from ref. 17, p. 441)} + 3.6 \text{ (from ref. 6)} - (d) \text{ kJ.mol}^{-1}$$
$$= 7.8 - (d) \text{ kJ.mol}^{-1}.$$

Since $\Delta G^0 = -RT \ln (N_Y/N_X)$, at 28° and at 0.72M concentration of **1**,

$$(d) = 7.8 - 5.9 \text{ kJ.mol}^{-1}.$$

Therefore, the anomeric effect of CO_2^- is ~ 1.9 kJ.mol^{-1}.

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