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D-Penturonic acids: solution studies of stable-isotopically enriched compounds by ^{1}H- and ^{13}C-n.m.r. spectroscopy

Jian Wu and Anthony S. Serianni*
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556 (U.S.A.)

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ABSTRACT

Methyl D-pentofuranosides were prepared by Fischer glycosidation of the aldopentoses D-arabinose, D-lyxose, D-ribose, D-xylose, and 2-deoxy-D-*erythro*-pentose, and oxidized with O_2 over a platinum oxide catalyst to give the corresponding methyl D-pentofuranosiduronic acids. After purification by anion-exchange chromatography, these glycosides were hydrolyzed to give the corresponding D-penturonic acids [D-arabinuronic acid (**1**), D-lyxuronic acid (**2**), D-riburonic acid (**3**), D-xyluronic acid (**4**), and 2-deoxy-D-*erythro*-penturonic acid (**5**)] in 80% yield based on the starting pentofuranoside. 1-^{13}C-Substituted D-aldopentoses were used to prepare D-(1-^{13}C)penturonic acids. Aqueous solutions of the 1-^{13}C-substituted penturonic acids, studied over a range of pH values by ^{13}C-n.m.r. spectroscopy, were found to contain α- and β-furanoses, acyclic aldehyde and hydrate, and/or hydrated 2,5-lactone. The ratio of D-riburonic acid anomers was most sensitive to solution pH (α/β = 0.49 and 1.2 at pH 1.9 and 4.9, respectively). The values of the ^{1}H and ^{13}C chemical shifts, and ^{1}H–^{1}H, ^{13}C–^{1}H, and ^{13}C–^{13}C spin-coupling constants, were determined by ^{1}H-(300, 500, and 620 MHz) and ^{13}C-(75 MHz) n.m.r. spectroscopy with the aid of 2-D ^{13}C–^{1}H chemical shift correlation maps, 2-D ^{1}H–^{1}H COSY data, and ^{13}C substitution, and were compared to those determined previously for structurally-related furanose rings. Isomerization of the penturonic acids at pH 5.0 and 50° gave the corresponding 4-pentulosonic acids.

INTRODUCTION

Uronic acids are constituents of biologically important glycosaminoglycans such as heparin (L-iduronic acid), hyaluronic acid, and chondroitin 4-sulfate (D-glucuronic acid) in vertebrates, and of plant pectic substances (D-galacturonic acid). Although the hexuronic acids occur more widely in nature, naturally occurring penturonic acids have also been reported; for example, D-riburonic acid appears to be a constituent of an extracellular polysaccharide synthesized by *Rhizobium meliloti*[1]. In contrast to the hexuronic acids, however, the solution properties of the penturonic acids have not been examined previously.

As part of our structure-reactivity studies of furanose sugars we have prepared five D-penturonic acids [D-arabinuronic acid (**1**), D-lyxuronic acid (**2**), D-riburonic acid (**3**), D-xyluronic acid (**4**), and 2-deoxy-D-*erythro*-penturonic acid (**5**)], the first four with ^{13}C substitution at C-1. The ^{1}H- and ^{13}C-n.m.r. spectra of **1**–**5** have been assigned, and

* Author for correspondence.

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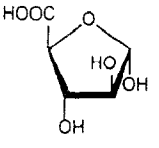
the tautomeric compositions of aqueous solutions of **1–4** have been examined as a function of solution pH by ^{13}C-n.m.r. spectroscopy. Values for the ^{1}H–^{1}H, ^{13}C–^{1}H, and ^{13}C–^{13}C spin-coupling constants were obtained from ^{1}H- (300, 500 and 620 MHz) and ^{13}C- (75 MHz) n.m.r. spectra, and these parameters were used to compare the conformational behavior of **1–4** to that reported previously for the 5-*O*-methylpentoses[2]. The isomerization of **1–4** to 4-pentulosonic acids has been studied, and the general application of D-penturonic acids as potential intermediates in the synthesis of stable-isotopically substituted carbohydrates is discussed.



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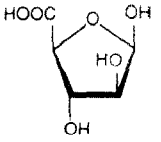

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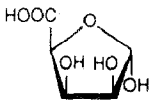

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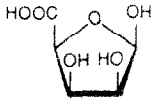

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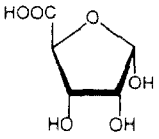

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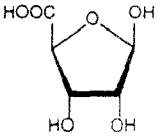

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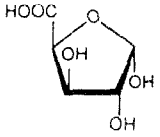

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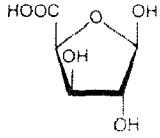

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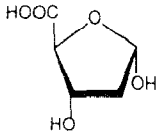

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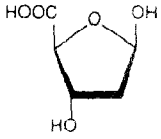

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EXPERIMENTAL SECTION

Materials. — D-Ribose, D-arabinose, D-xylose, D-lyxose, 2-deoxy-D-*erythro*-pentose, D-glucuronic acid, Dowex ion-exchange resins, DEAE-Sephadex A-25 (40–120 mesh), and Amberlite IRA-68 (16–50 mesh) resin were purchased from Sigma Chemical Co. Platinum oxide, platinum on activated carbon (5%), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% chlorotrimethylsilane (TMCS) were purchased from Aldrich Chemical Co. Potassium (^{13}C)cyanide [K^{13}CN, 99 at.% ^{13}C] and deuterium oxide ($^{2}H_2O$, 98 at.% ^{2}H) were obtained from Cambridge Isotope Laboratories. D-Erythrose and D-threose were prepared from 4,6-*O*-ethylidene-D-glucose[3] and 4,6-*O*-ethylidene-D-galactose[4], respectively. Methyl β-D-(2-^{13}C)ribofuranoside was obtained from Mr. Paul Kline, Department of Chemistry and Biochemistry, University of Notre Dame.

D-(1-^{13}C)Pentoses were synthesized from the parent tetroses (D-erythrose and

D-threose) and $K^{13}CN$ by the cyanohydrin reduction method[5,6] and purified by chromatography on Dowex 50-X8 (200–400 mesh) resin in the calcium form[7].

Instrumentation. — Broad-band ^1H-decoupled ^{13}C-n.m.r. spectra (75 MHz) and ^1H-n.m.r. spectra (300 MHz) were obtained at room temperature (~20°) on Nicolet NT-300 or General Electric GN-300 300-MHz F.t.-n.m.r. spectrometers equipped with quadrature-phase detection and 293B pulse programmers. ^1H-N.m.r. spectra at 500 MHz were obtained on a Varian VXR-500S F.t.-n.m.r. spectrometer located in the College of Science High-Field NMR Facility at the University of Notre Dame. ^{13}C-N.m.r. spectra were referenced to sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) by measurement from the C-1 signal of external α-D-(1-^{13}C)mannopyranose (95.5 p.p.m.), and ^1H-n.m.r. spectra were referenced internally to DSS. Values for ^{13}C and ^1H chemical shifts are accurate to ±0.1 p.p.m. and ±0.01 p.p.m., respectively, and spin-coupling constants are accurate to ±0.1 Hz.

F.t.-n.m.r. spectra (^1H) at 620 MHz were obtained at the NMR Facility for Biomedical Studies, Department of Chemistry, Carnegie Mellon University.

Two-dimensional ^{13}C–^1H chemical shift correlation spectra were obtained on the GN-300 n.m.r. spectrometer using a revised GN software program CSCMR; 64 blocks of 2056 data points were collected per spectrum. Two-dimensional ^1H–^1H homonuclear shift correlation (COSY) spectra (absolute-value) were obtained on the same spectrometer using standard GN software; 512 blocks of 1024 data points were collected per spectrum.

Gas-liquid chromatography–mass spectrometry (g.l.c.-m.s.) of the per-*O*-trimethylsilylated derivatives of D-penturonic acids **1–5** was performed on a Finnigan-MAT 8430 mass spectrometer coupled to a Varian 3400 gas chromatograph and operated in the positive-ion chemical ionization mode with ammonia as the reagent gas. An aqueous sample (100 μL) containing 100 μg of the D-penturonic acid (free acid) was mixed with pyridine (150 μL) in a small vial, BSTFA (200 μl) containing 1% TMCS was added, and the vial was sealed with a Teflon-lined cap and heated for 30 min at 70°. The α and β anomers of the derivatized penturonic acids were separated on a Supelco SPB-1 gas-liquid chromatographic capillary column (0.32 mm i.d. × 15 m), with the temperature programmed from 35 to 320° at 20°/min after isothermal operation for 1 min at 35°. The quasimolecular ion $(M + 1)^+$ occurring at *m/z* 453 was used to characterize the penturonic acids; this value increased by 1 amu for the 1-^{13}C-substituted compounds.

Methyl glycosidation of D-*pentoses.* — D-Arabinose, D-xylose, or D-lyxose (1.5 g, 10 mmol) was dissolved in 50 mL of anhydrous methanol and the solution was concentrated to a syrup at 30° *in vacuo*; this process was repeated twice more to remove residual water. The resulting syrup was dissolved in anhydrous methanol (60 mL) and concentrated sulfuric acid (0.25 mL) was added with efficient stirring. After 24 h at room temperature (~23°), the reaction mixture was applied to a chromatographic column (2.8 × 23 cm) containing excess Amberlite IRA-68 (OH^-) resin to neutralize the acid. The column was washed with anhydrous methanol until a negative phenol–sulfuric acid assay[8a] was obtained. The neutral effluent was concentrated to ~5 mL at 30° *in vacuo*, and loaded on a chromatographic column (2.8 × 60 cm) containing Dowex 1-X2

(OH^-) resin (200–400 mesh)[8b]. The column was eluted with CO_2-free distilled water at a flow rate of 1 mL/min, and fractions (18 mL) were collected and assayed with phenol-sulfuric acid[8a]. The elution order of the glycosides (furanosides and pyranosides) and the proportions present after glycosidation are given in Tables I and II, respectively. Fractions containing the purified glycosides were pooled and concentrated to ~10 mL at 35° *in vacuo*.

For the preparation of methyl D-ribofuranosides[9a], D-ribose (1.5 g, 10 mmol) was dissolved in 30 mL of anhydrous methanol at 4°, concentrated sulfuric acid (0.15 mL) was added, and the reaction mixture was incubated at 4° for 12–16 h. The furanosides were purified as just described. For the preparation of methyl 2-deoxy-D-*erythro*-pentofuranosides, 2-deoxy-D-*erythro*-pentose (1.3 g, 10 mmol) was dissolved in 0.5% v/v HCl in methanol (100 mL). After 6 min at 23°, the acidic solution was neutralized by the batchwise addition of Amberlite IRA-68 (OH^-) resin (80 g), and the glycosides were purified as described above.

Catalytic oxidation of methyl D-*pentofuranosides to methyl* D-*pentofuranosiduronic acids.* — Methyl α- or β-D-pentofuranoside (5 mmol) was dissolved in 100 mL of distilled water (pH ~7.5) in a side-arm vacuum flask, and sodium hydrogencarbonate (~100 mg) was added. Platinum oxide (150 mg), or 5% platinum on activated carbon (300 mg), that had been pre-reduced with hydrogen, was added, and the reaction flask was placed in an oil bath at 50°. Oxygen was bubbled through the suspension at a rate of ~250 mL/min, and the mixture was stirred magnetically. As the production of acids proceeded, the solution pH dropped from an initial value of 8–9, but was maintained above 7 by occasional additions of sodium hydrogencarbonate (80 mg), to facilitate the reaction and prevent glycoside hydrolysis. The reaction was judged complete when no further drop in pH was noted (7–11 h).

After completion of the reaction the catalyst was removed by vacuum filtration through a Whatman GF/B filter and Millipore AW prefilter. The reaction mixture was applied to a column (2.8 × 35 cm) of DEAE-Sephadex A-25 in the hydrogencarbonate form, and the components were eluted with a 3000 mL linear gradient (0.01–0.08M) of sodium hydrogencarbonate (pH 8.5) at a flow rate of 0.8 mL/min. Fractions (18 mL) were assayed with phenol-sulfuric acid[8a]. In each case fractions ~8–18 contained unreacted methyl D-pentofuranoside and fractions ~60–95 contained the product methyl pentofuranosiduronates. The latter fractions were pooled and concentrated at 30° *in vacuo* to ~10 mL.

Hydrolysis of methyl D-*pentofuranosiduronic acids to* D-*penturonic acids.* — Aqueous solutions of purified methyl α- or β-D-pentofuranosiduronates (5 mmol in 100 mL of water) were treated with Dowex HCR-W2 (H^+) ion-exchange resin (50 g) for 7–8h at room temperature with magnetic stirring; this treatment also removed hydrogencarbonate, which was lost as CO_2. The resin was removed by vacuum filtration, yielding an acidic filtrate containing the D-penturonic acid (free acid), as assayed by ^{13}C-n.m.r. spectroscopy. The hydrolysis of methyl α-D-lyxofuranosiduronic acid was conducted for 7–8 h at 50°.

*Reduction of methyl β-*D-*ribofuranosiduronic acid to methyl β-*D-*ribofuranoside.*

Methyl β-D-ribofuranosiduronic acid (5 mmol) was dissolved in 150 mL of water, the solution was adjusted to pH ~4.7 with M HCl, and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (10 mmol) was added. Since the reaction consumes hydrogen ion, the solution pH was maintained at ~4.7 with periodic additions of 0.1M HCl. The reaction was allowed to proceed until H^+ uptake ceased. Sodium borohydride (50 mL of a M aqueous solution) was then added dropwise at room temperature while maintaining the solution pH at 7.0 with M HCl, and the mixture was heated for 2 h at 50° with stirring. The solution was cooled in an ice bath and the excess borohydride was destroyed by dropwise addition of 1.5M H_2SO_4 until hydrogen evolution ceased. The resulting solution was cooled at 4° overnight and filtered (vacuum) through Whatman GF/B filter paper. The filtrate was treated batchwise with Dowex 1-X8 resin in the hydrogencarbonate form, concentrated to ~5 mL at 35° *in vacuo*, and applied to a column (2.8 × 30 cm) containing Dowex 50-X8 (200–400 mesh) resin in the calcium form[7]. The column was eluted with distilled water at a flow rate of 1 mL/min, and fractions (18 mL) were collected and assayed with phenol–sulfuric acid[8a]. Fractions 12–20 were pooled and concentrated at 30° *in vacuo* to a syrup, which was identified as methyl β-D-ribofuranoside by its characteristic ^{13}C-n.m.r. chemical shifts[9c]. The yield was 0.74 g, 4.5 mmol, 90%.

Base-catalyzed isomerization of D-*penturonic acids.* — Aqueous solutions (0.2M) of D-(1-^{13}C)penturonic acid and D-(2-^{13}C)riburonic acid (10 mL) were adjusted to pH 5.0 with NaOH and incubated for 48 h at 50°. The resulting solutions were assayed by ^{13}C-n.m.r. spectroscopy.

RESULTS AND DISCUSSION

The synthesis of the D-penturonic acids **1–5** involves the preparation of the appropriate methyl D-pentofuranosides and their catalytic oxidation at C-5 with O_2. The glycosidation of D-pentoses was examined using HCl (0.4M) or H_2SO_4 (75mM) as the acid catalyst; the latter consistently gave better yields of pentofuranosides. The separation of pentofuranoside anomers was achieved by chromatography on Dowex 1-X8 (200–400 mesh) anion-exchange resin in the hydroxide form[8b] (Table I).

The oxidation of the primary alcohol groups of suitably protected sugars over a platinum catalyst has been reported previously[10,11]. Platinum-catalyzed oxidation of the methyl D-pentofuranosides (α or β anomers) was highly selective for the primary alcohol functionality at C-5, giving the corresponding methyl D-pentofuranosiduronate salt in high yield (Fig. 1A,B), and this was separated from the unreacted pentofuranoside by chromatography on DEAE-Sephadex in the hydrogencarbonate form (Fig. 1C). The overall yield (~80%) of penturonic acid from methyl D-pentofuranoside is acceptable for the preparation of ^{13}C-substituted compounds.

Taylor *et al.*[12a] have reported that the carboxyl group of uronic acids in polysaccharides can be activated by a carbodiimide and reduced to the alcohol with sodium borohydride. This strategy was used to reduce methyl β-D-ribofuranosiduronic acid, in good yield, to methyl β-D-ribofuranoside. Hence, this method may be applied with

TABLE I

Separation of methyl D-pentofuranosides and -pentopyranosides by chromatography on Dowex 1-X2 (200–400 mesh) resin in the hydroxide form[a]

Starting pentose	*Elution order of glycosides*[b]
D-Arabinose	α,β-pyranoside (25–50); β-furanoside (62–80); α-furanoside (140–200)
2-Deoxy-D-*erythro*-pentose	α-furanoside (25–40); β-furanoside (43–75)
D-Lyxose	α,β-pyranoside (17–35); β-furanoside (38–45); α-furanoside (150–220)
D-Ribose	α-furanoside (35–65); β-furanoside (80–140)
D-Xylose	α,β-pyranoside (26–40); α-furanoside (42–70); β-furanoside (72–100)

[a] Column dimensions and elution conditions are given in the text. [b] Values in parentheses are fraction numbers defining the elution volumes of the successive glycosides, which were identified by their characteristic ^{13}C chemical shifts[9b].

sodium borodeuteride as the reducing agent to afford methyl (5-$^2H_2$)pentofuranosides.

(1-^{13}C)Penturonic acids may serve as useful precursors in the synthesis of multiply ^{13}C-labeled aldopentoses and aldohexoses. For example, D-(1-^{13}C)lyxuronic acid could be reduced with $NaBH_4$ to yield D-(5-^{13}C)arabinonic acid[12b] (**6**), and subsequent γ-lactonization, reduction, carbon addition[5,6], and molybdate epimerization[12c] should give D-(1,6-$^{13}C_2$)glucose and D-(2,6-$^{13}C_2$)glucose.

Assignment of ^{13}C and ^1H chemical shifts. — The ^{13}C-n.m.r. signals of furanose ring carbons are affected in a predictable fashion by the relative configurations of the hydroxyl groups attached to these carbons. Contiguous ring carbons bearing *cis* hydroxyl groups have ^{13}C signals ~5.0 p.p.m. upfield of those for similar carbons bearing *trans* hydroxyl groups[9c]. Using this rule, the C-1 chemical shifts were assigned to specific furanose species; thus, the downfield C-1 signals in **1–4** were assigned to the α-*arabino* (**1a**), α-*lyxo* (**2a**), β-*ribo* (**3b**), and β-*xylo* (**4b**) anomers (Table III). Using these C-1 assignments, 2-D ^{13}C-^1H chemical shift correlation maps (for D-riburonic acid, see Fig. 2) were obtained on **1–4** to assign the anomeric (H-1) protons. The H-2, H-3, and H-4 signals were identified from ^1H–^1H COSY spectra (Fig. 3, Table IV), and the C-2, C-3, and C-4 signals were assigned *via* 2-D ^{13}C–^1H chemical shift correlation spectroscopy. The C-2 signal assignments were confirmed by the observed splitting of these signals in 1-^{13}C-enriched compounds, which show a large one-bond ^{13}C–^{13}C spin-coupling constant (~45 Hz) (Table V). The C-5 signals were assigned from relative signal intensities, and are therefore reported with less certainty.

A similar strategy was employed to assign the carbon signals of several methyl pentofuranosiduronic acids (Table VI).

The ^1H chemical shifts of 2-deoxy-D-*erythro*-penturonic acid (**5**) were assigned *via* 2-D ^1H–^1H COSY data (Table IV). For the α-furanose (**5a**), H-2*S* is *cis* to H-1 and H-3, while H-2*R* is *cis* to O-1 and O-3. The "syn-upfield rule"[13a,b] predicts that H-2*R* will be more shielded than H-2*S*. In contrast, in the β-furanose (**5b**), H-2*R* and H-2*S* are each

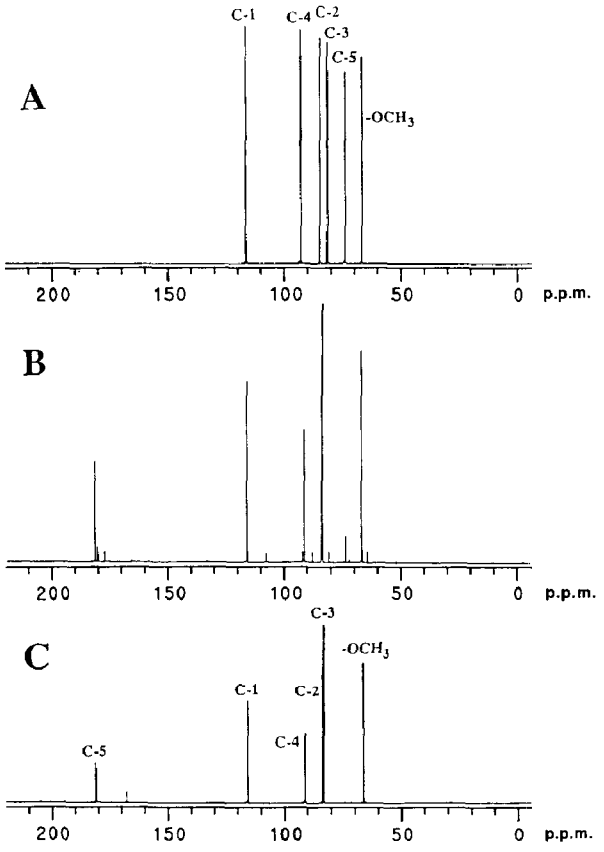


Fig. 1. *A*, The ^{1}H-decoupled ^{13}C-n.m.r. spectrum (75 MHz) of methyl β-D-ribofuranoside, showing signal assignments; *B*, the ^{1}H-decoupled ^{13}C-n.m.r. spectrum (75 MHz) of the reaction mixture after oxidation of this glycoside over Pt (from PtO_2), showing high conversion to methyl β-D-ribofuranosiduronate with minimal formation of byproducts; *C*, the ^{1}H-decoupled ^{13}C-n.m.r. spectrum (75 MHz) of methyl β-D-ribofuranosiduronate after purification by chromatography on DEAE-Sephadex (HCO_3^-). The small signal at ~168 p.p.m. is due to residual hydrogencarbonate.

TABLE II

Proportions of anomers in the glycosidation of D-pentoses

Pentose	*Percentage in reaction mixture*[a]				
	α-f	β-f	α-p	β-p	*Unreacted sugar*
D-Arabinose	65	25	5	5	0
2-Deoxy-D-*erythro*-pentose	44	54	nd	nd	2
D-Lyxose	66	9	5	17	3
D-Ribose	25	75	nd	nd	nd
D-Xylose	43	50	2	4	1

[a] α-f = α-furanoside, β-f = β-furanoside, α-p = α-pyranoside, β-p = β-pyranoside; nd = not detected. Determined by integration of C-1 signals in ^{13}C-n.m.r. spectra of reaction mixtures prior to chromatography on Dowex 1(OH^-) (see text).

TABLE III

Carbon-13 chemical shifts of the D-penturonic acids in ^2H_2O

Compound	*Chemical shift (p.p.m.)*[a]				
	C-1	*C-2*	*C-3*	*C-4*	*C-5*
α-D-Arabinuronic acid (**1a**)	103.9 (102.8)	81.3 (82.9)	80.0 (77.7)	83.2 (83.0)	175.4
β-D-Arabinuronic acid (**1b**)	98.2 (96.9)	76.9 (77.7)	78.4 (76.2)	80.4 (80.9)	176.0
2-Deoxy-α-D-*erythro*-penturonic acid (**5a**)	100.5	41.5	75.0	84.6	175.6
2-Deoxy-β-D-*erythro*-penturonic acid (**5b**)	100.9	41.9	75.0	84.2	176.0
α-D-Lyxuronic acid (**2a**)	102.5 (101.3)	77.6 (77.6)	72.9 (71.8)	80.0 (78.7)	173.9
β-D-Lyxuronic acid (**2b**)	97.7 (96.0)	72.2 (72.0)	71.9 (70.7)	80.6 (78.9)	174.1
α-D-Riburonic acid (**3a**)	98.2 (97.9)	71.8 (72.3)	74.2 (71.8)	81.4 (82.6)	175.6
β-D-Riburonic acid (**3b**)	103.1 (102.8)	76.2 (76.7)	74.6 (72.4)	80.8 (82.0)	176.2
α-D-Xyluronic acid (**4a**)	98.6 (95.8)	76.5 (76.4)	76.9 (75.4)	79.4 (76.9)	174.4
β-D-Xyluronic acid (**4b**)	104.3 (102.1)	80.6 (80.8)	76.7 (75.3)	82.5 (80.0)	174.3

[a] Chemical shifts were measured on compounds as their free acids (pH 1.6), are referenced to the anomeric carbon signal of α-D-(1-^{13}C)mannopyranose (95.5 p.p.m., see text), and are accurate to ± 0.1 p.p.m. Values in parentheses are chemical shifts in the corresponding 5-*O*-methylpentoses[2].

cis to one hydrogen and one hydroxyl group, and similar chemical shifts are expected. This predicted chemical shift behavior of the C-2 methylene protons was used as a starting point to assign the H-1, H-3, and H-4 signals of each anomer of **5** *via* the COSY spectrum. The ^{13}C chemical shifts could then be assigned *via* a 2-D ^{13}C–^{1}H chemical shift correlation map (Table III).

Of the eight furanose anomers of **1–4**, examined at pH 1.6, five give a similar chemical shift pattern for the *ring* carbons: $\delta_{C-1} > \delta_{C-4} > \delta_{C-2} > \delta_{C-3}$; the exceptions are **1b**, **3a**, and **4a** in which $\delta_{C-3} > \delta_{C-2}$. A similar pattern was found previously in seven of eight 5-*O*-methyl-D-pentofuranose anomers[2]. The exocyclic carboxyl carbon in **1–4** resonates at 174–176 p.p.m. at pH 1.6. Hydrogen chemical shift data (Table IV) show H-1 and H-2 to be the most deshielded and most shielded protons, respectively, in **1–4** at pH 1.6; in most but not all cases, $\delta_{H-4} > \delta_{H-3}$. Similar behavior was noted previously in most of the 5-*O*-methyl-D-pentofuranoses[2].

Effect of solution pH on ^{13}*C* chemical shifts. The ^{13}C chemical shifts for D-riburonic acid were determined as a function of solution pH (Fig. 4). The magnitude of change in δ decreases in the following order: C-5 > C-4 > C-3 ≈ C-2 > C-1. Thus, the sensitivity of ^{13}C chemical shift to the state of carboxyl ionization in pentofuranuronic acids decreases as the carbon in question is further removed from the site of ionization. The C-2–C-5 resonances shift downfield with increasing pH, whereas the C-1 signal shifts upfield. Similar behavior has been observed in D-galacturonic and D-glucuronic acids[14a]. From plots of $\Delta\delta$ *vs.* pH (Fig. 4), an average pK_a of ~2.9 was estimated for D-riburonic acid; the pK_a of the α-anomer (**3a**) appears to be slightly lower than that of the β-anomer (**3b**).

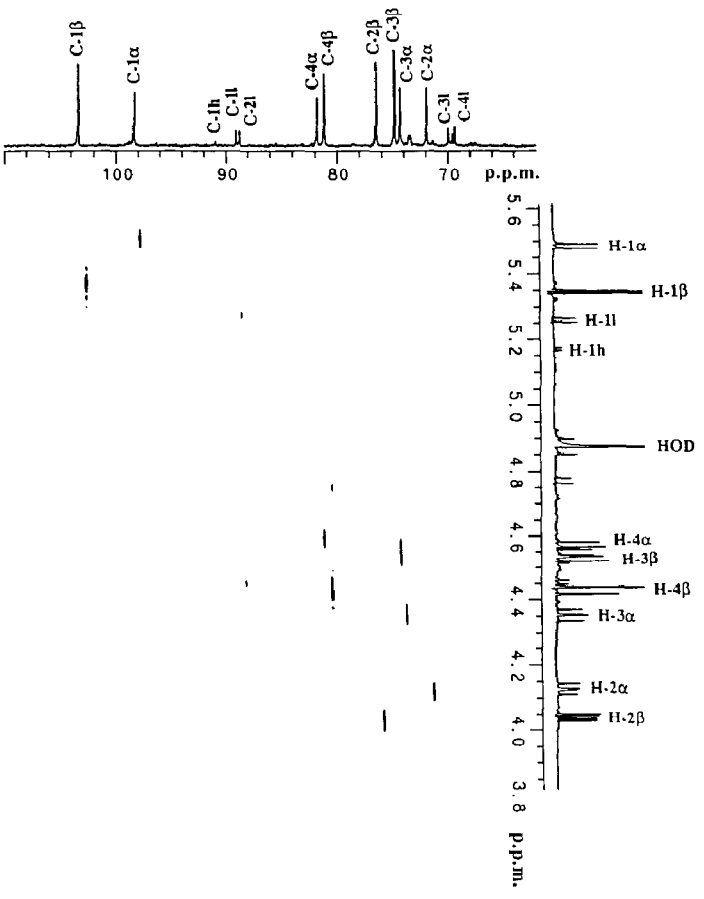


Fig. 2. The 2-D ^{13}C–^{1}H chemical shift correlation map of D-riburonic acid (free acid) in $^{2}H_2O$. Signal assignments are shown in the 1-D ^{13}C- and ^{1}H-n.m.r. spectra along the axes; h = acyclic hydrate, l = 2,5-lactone hydrate. Note the significantly different correlation signals for the two closely spaced carbon signals at ~89 p.p.m., which allows the assignment of the signal at 88.8 p.p.m. to C-1, and that at 88.6 p.p.m. to C-2, of the 2,5-lactone (see text).

Solution composition of the D-*penturonic acids.* — In aqueous solution, the D-penturonic acids **1–4** may exist in several interconverting forms (Scheme 1). The monomeric forms include the α- and β-furanoses, acyclic aldehyde, acyclic hydrate (1,1-*gem*-diol), 2,5-lactone-aldehyde, and 2,5-lactone hydrate (Fig. 5A, Scheme 1). Intramolecular 2,5-lactonization is possible in isomers having O-2 *cis* to C-5 (*e.g.* **1** and **2**), forming bicyclic products.

The tautomeric compositions of aqueous solutions of the D-(1-^{13}C)penturonic acids were determined from ^{13}C-n.m.r. spectra obtained at pH 1.6 (Table VII). The most abundant forms in aqueous solution are the furanoses (79–93%); in **1**, **2**, and **3** the more stable furanose anomer has O-1 and O-2 *trans*, whereas the more stable anomer of **4** has O-1 and O-2 *cis*. Thus, in their protonated states, the anomeric proportions of the

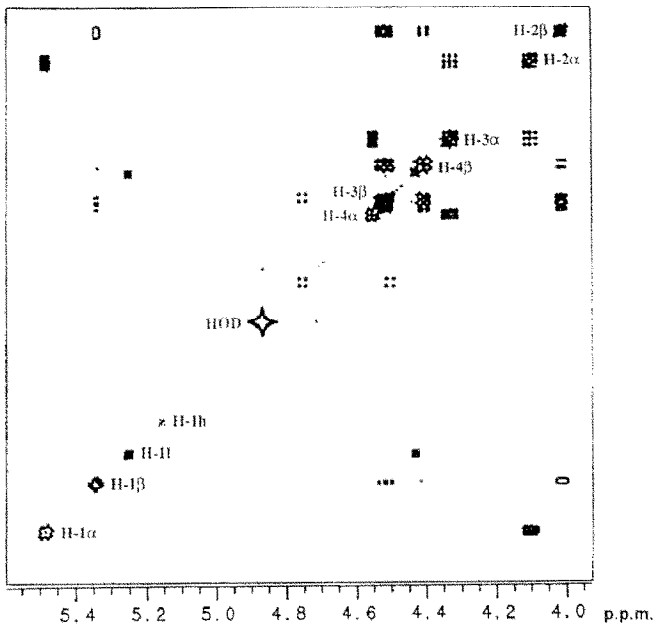


Fig. 3. The 2-D ^1H-^1H COSY spectrum of D-riburonic acid (free acid) in ^2H_2O, showing signal assignments along the diagonal; h = acyclic hydrate, l = 2,5-lactone hydrate.

TABLE IV

^1H Chemical shifts of the D-penturonic acids in ^2H_2O

Compound	*Chemical shift (p.p.m.)*[a]					
	H-1	*H-2*	*H-2*R	*H-2*S	*H-3*	*H-4*
α-D-Arabinuronic acid (**1a**)	5.41 (5.23)	4.08 (4.02)			4.35 (3.93)	4.69 (4.17)
β-D-Arabinuronic acid (**1b**)	5.44 (5.28)	4.10 (4.07)			4.43 (4.00)	4.37 (3.89)
2-Deoxy-α-D-*erythro*-penturonic acid (**5a**)	5.69		1.95	2.34	4.57	4.65
2-Deoxy-β-D-*erythro*-penturonic acid (**5b**)	5.72		2.13[b]	2.18[b]	4.88	4.43
α-D-Lyxuronic acid (**2a**)	5.40 (5.28)	4.11 (4.08)			4.55 (4.32)	4.92 (4.41)
β-D-Lyxuronic acid (**2b**)	5.37 (5.26)	4.22 (4.17)			4.51 (4.26)	4.72 (4.17)
α-D-Riburonic acid (**3a**)	5.50 (5.36)	4.15 (4.09)			4.38 (obs)	4.61 (obs)
β-D-Riburonic acid (**3b**)	5.37 (5.23)	4.06 (3.98)			4.57 (4.17)	4.46 (4.04)
α-D-Xyluronic acid (**4a**)	5.61 (5.40)	4.15 (4.07)			4.53 (4.29)	4.89 (4.37)
β-D-Xyluronic acid (**4b**)	5.36 (5.20)	4.18 (4.06)			4.46 (4.19)	4.90 (4.34)

[a] Chemical shifts were measured on compounds as their free acids (pH 1.6), are relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS), and are accurate to ± 0.01 p.p.m. Values in parentheses are chemical shifts in the corresponding 5-*O*-methylpentoses[2]; "obs" denotes obscured signals. [b] Assignments may be reversed.

TABLE V

Values for some ^{1}H–^{1}H and ^{13}C–^{13}C coupling constants in the D-penturonic acids[a]

Compound	*Coupled nuclei*					
	H-1,H-2	*H-2,H-3*	*H-3,H-4*	*C-1,C-2*	*C-1,C-3*	*C-1,C-5*
α-D-Arabinuronic acid (**1a**)	1.8 (2.7)	2.9 (4.6)	3.9 (6.5)	46.1 (46.4)	2.8 (4.2)	2.2 (br.)
Methyl α-arabinofuranosiduronic acid	0.9	2.1	4.5	46.9	1.6	2.3
β-D-Arabinuronic acid (**1b**)	4.3 (4.5)	6.3 (7.4)	5.7 (7.3)	43.5 (43.7)	3.8 (3.1)	
α-D-Lyxuronic acid (**2a**)	5.4 (4.4)	4.8 (~4.5)	3.9 (~4.3)	46.3 (46.6)	2.1 (2.2)	2.6 (2.2)
Methyl α-D-lyxofuranosiduronic acid	4.2	4.7	3.7	46.9	1.9	3.0
β-D-Lyxuronic acid (**2b**)	5.1 (5.0)	5.1 (~4.8)	4.5 (4.3)	43.7 (43.0)		
α-D-Riburonic acid (**3a**)	4.1 (4.0)	5.3 (5.7)	4.8	42.5 (42.5)	2.2 (2.3)	br. (2.0)
α-D-Riburonate (**3a**) (pH 4.7)	4.5	5.4	3.2	42.9		
β-D-Riburonic acid (**3b**)	1.7 (1.6)	4.5 (4.7)	6.3 (6.8)	46.5 (46.1)	2.6 (3.3)	br.
β-D-Riburonate (**3b**) (pH 4.7)	3.0	4.8	5.3	46.0	3.2	
Methyl β-ribofuranosiduronic acid	2.1	4.6	6.1	46.8	2.7	
α-D-Xyluronic acid (**4a**)	3.7 (4.3)	3.4 (4.5)	5.2 (5.4)	40.7 (42.5)		3.2 (~0)
β-D-Xyluronic acid (**4b**)	1.0 (1.4)	1.8 (2.2)	5.0 (4.8)	45.3 (45.5)		

[a] Reported in Hz, accurate to ±0.1 Hz. No entry means that coupling was not observed; "br." denotes broadened signals. Values in parentheses are couplings in the corresponding 5-*O*-methylpentoses[2].

TABLE VI

Carbon-13 chemical shifts for methyl D-pentofuranosiduronate ions at pH 7.5

Methyl pentofuranosiduronate	*Chemical shift (p.p.m.)*[a]					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*OCH_3*
α-D-*arabino*	110.3	81.6	81.2	85.4	178.8	56.6
2-Deoxy-β-D-*erythro*	107.7	41.2	75.2	86.4	178.9	56.9
α-D-*lyxo*	110.0	77.6	73.4	82.7	176.9	57.4
β-D-*ribo*	109.9	75.7	75.2	83.7	179.8	57.4
β-D-*xylo*	110.9	81.0	77.6	85.0	177.5	57.9

[a] Chemical shifts are referenced to the anomeric carbon signal of α-D-(1-^{13}C)mannopyranose (95.5 p.p.m., see text) and are accurate to ±0.1 p.p.m.

D-penturonic acids are similar to those found for the 5-*O*-methylpentoses (Table VII) and 5-deoxypentoses[2]. The acyclic aldehyde is present in low abundance (<0.2%), but was identified by its characteristic C-1 chemical shift (~206 p.p.m.)[14b].

In addition to the furanose and acyclic aldehyde forms, aqueous acidic solutions

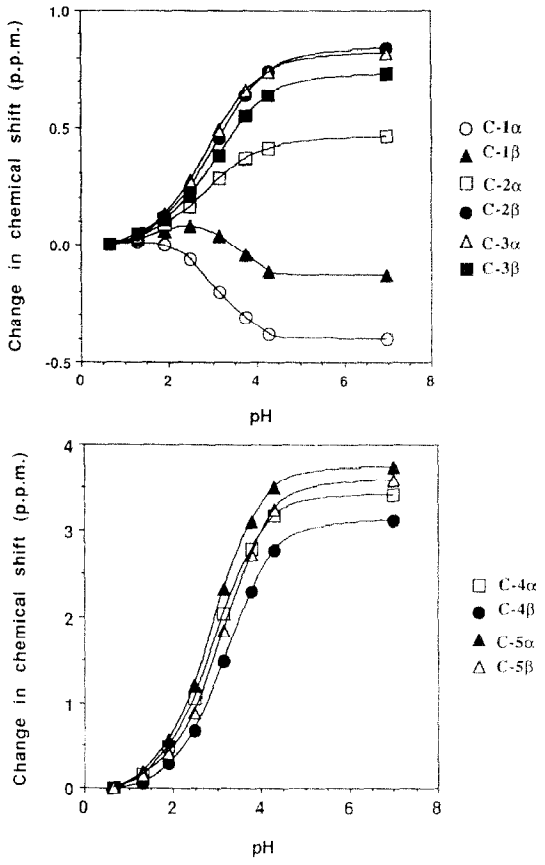
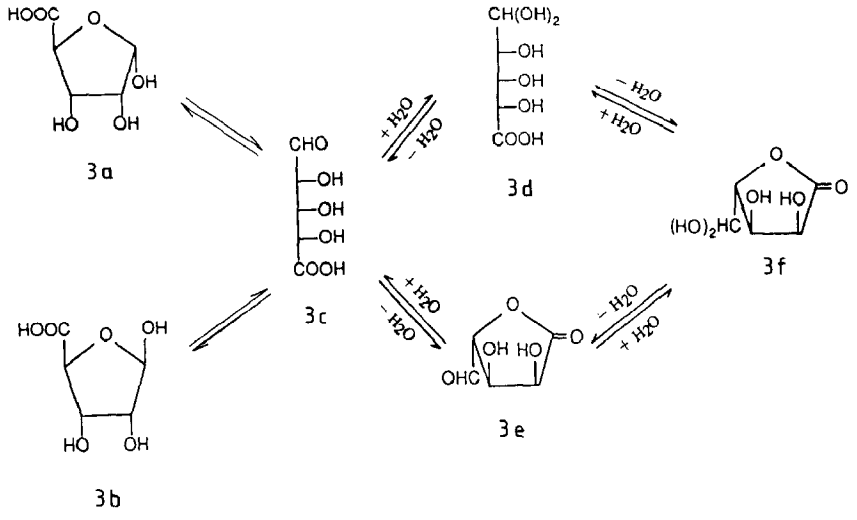


Fig. 4. The effect of solution pH on the ^{13}C chemical shifts of the α- and β-furanose forms of D-riburonic acid.

of **1–4** contain minor amounts of acyclic hydrate and/or 2,5-lactone hydrate (Table VII). For example, 2,5-lactonization of the acyclic aldehyde **3c** or hydrate **3d** gives γ-lactones **3e** and **3f** having the L-*ribo* configuration (Scheme 1). The ^{13}C-n.m.r. spectrum of **3** at pH 1.6 contained signals at 88.6, 69.8, 69.2, and 179.7 p.p.m., which were assigned to C-2, C-3, C-4, and C-5, respectively, of **3f**, since these chemical shifts are similar to those found for D-ribono-1,4-lactone (88.3, 71.1, 70.6, and 180.0 p.p.m. for C-4, C-3, C-2, and C-1, respectively) by Angelotti *et al.*[15a]. The exocyclic C-1 carbon of the putative lactone resonated at 88.8 p.p.m., indicating that the aldehyde group is hydrated. Although δ_{C-1} (88.8 p.p.m.) and δ_{C-2} (88.6 p.p.m.) were very similar, the signal at 88.8 p.p.m. correlated with a proton in the anomeric region of the 2-D ^{13}C-^{1}H shift correlation spectrum (Fig. 2), confirming its assignment to C-1. The ^{13}C-n.m.r. spectrum of D-(2-^{13}C)riburonic acid also gave an enriched signal at 88.6 p.p.m., and provided supportive evidence for the presence of **3f** in aqueous solutions of **3**.

The proportion of 2,5-lactone hydrate is high (~10%) in aqueous solutions of D-riburonic acid (free acid), but decreases with increasing pH, becoming undetectable at



Scheme 1

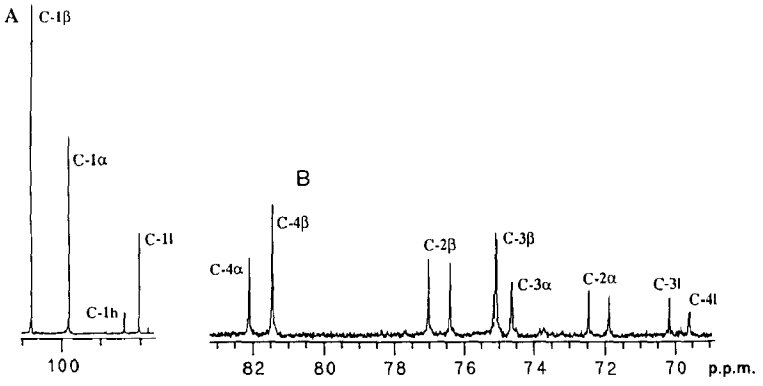


Fig. 5. *A*, The anomeric region of the ^{1}H-decoupled ^{13}C-n.m.r. spectrum (75 MHz) of D-(1-^{13}C)riburonic acid (**3**), showing the enriched C-1 carbons of the α-furanose, β-furanose, acyclic hydrate (h), and lactone hydrate (l) forms; *B*, spectrum of the unenriched carbons of **3**, showing signal assignments to the α- and β-furanose and lactone forms, and the presence of ^{13}C–^{13}C spin coupling.

neutrality. This behavior is consistent with that of aldonolactones, which hydrolyze in alkaline solutions. In contrast to the findings for **1–4**, lactones were not detected in aqueous acidic solutions of 2-deoxy-D-*erythro*-penturonic acid (**5**), which lacks the hydroxyl group at C-2 required for 2,5-lactonization; only α- and β-furanose, acyclic hydrate, and aldehyde forms were observed. It is noteworthy that, in **5**, the α-furanose is more abundant than the β-furanose (Table VII); similar behavior has been observed in aqueous solutions of 5-*O*-methyl-2-deoxy-D-*erythro*-pentose[15b].

Effect of pH on the solution composition of D-*penturonic acids.* — Solution pH, and thus the ionization state of the carboxyl group, appears to affect the relative proportions

TABLE VII

Proportions of the interconverting forms of the D-penturonic acids in solutions of the free acids[a]

Compound	*Percent of total*[b]				
	α-f	β-f	Acyclic hydrate	Lactone hydrate	α/β
D-Arabinuronic acid (**1**)	55 (60)	38 (37)	3 (3)	4	1.4 (1.6)
2-Deoxy-D-*erythro*-penturonic acid (**5**)	53 (52)	42 (45)	5	0	1.3 (1.2)
D-Lyxuronic acid (**2**)	58 (72)	21 (25)	10 (3)	11	2.8 (2.9)
D-Riburonic acid (**3**)	35 (35)	57 (65)	2 (1)	6	0.62 (0.53)
D-Xyluronic acid (**4**)	56 (55)	36 (41)	5 (4)	3	1.6 (1.3)

[a] pH ~1.6, 23°, 3:1 (v/v) ^2H_2O:H_2O. [b] α-f = α-furanose, β-f = β-furanose; values in parentheses are percentages for the corresponding forms of the 5-*O*-methylpentoses[7].

of furanose anomers in aqueous D-riburonic acid. As shown in Fig. 6, β-D-ribo-furanuronic acid (**3b**) predominates at pH values < 1.8 (27% **3a**, 57% **3b**). As solution pH increases, the percentages of the α- and β-furanoses increase and decrease to values of 55% and 46%, respectively, at pH 5. In the protonated state, **3b** is more stable than the α anomer **3a** (paralleling the behavior of neutral furanoses having the *ribo* configuration[2]), whereas in the ionized state, **3a** predominates. In addition, the proportion of 2,5-lactone hydrate **3f** decreases with increasing pH (~11% at pH 0.5, undetectable at pH 5.0) (data not shown).

The effect of change in pH on the relative stabilities of furanose anomers appears to be greatest in **3**. In **1**, a smaller effect is observed (Fig. 6), whereas essentially no effect is found in **2** and **4**. Apparently, the presence of one or more hydroxyl groups *cis* to C-5 reduces or eliminates the pH effect. By comparison, solution pH (1.5–5.0) had little effect on the ratio of α- and β-furanoses in the D-pentofuranose 5-phosphates[16,17]. Interestingly, aqueous solutions of D-glucuronic acid at pH 1.4 contain 48% α-pyranose and 52% β-pyranose, whereas at pH 4.0, the proportions are 41% and 59%, respectively[16]. Thus, in contrast to **3**, the solution composition of the *ionized* form of D-glucuronic acid is similar to that found for neutral D-glucose, which exists as ~40% α-pyranose and ~60% β-pyranose in aqueous solution[17].

The factors responsible for the change in the relative stabilities of D-pento-furanuronic acid anomers with change in pH are not obvious, especially since the effect is essentially confined to **3**. Inorganic cations have been shown to modify the equilibrium proportions of furanose anomers in solution. For example, furanoses having three hydroxyl groups oriented *cis* on contiguous carbons (*e.g.*, α-*ribo* isomers) form strong complexes with calcium[18]. Although the observed effects of pH on the anomeric proportions of **3** might be attributed to differential cation complexation, this explanation is unlikely since divalent cations were not employed in the titrations and repeated forward and reverse titrations on the same sample using monovalent cations yielded the same results.

An alternative explanation may be that, in **3**, solution pH has a significant effect on the ring conformations of one or both anomers, causing their relative stabilities to change. ^{1}H-N.m.r. spectra of **3** obtained at pH 1.4, 3.3, 3.9, and 4.7 showed that $^3J_{H\text{-}1,H\text{-}2}$ increased from 1.7 Hz to 3.0 Hz for **3b**, and from 4.1 Hz to 4.5 Hz in **3a**. Over the same pH range, $^3J_{H\text{-}2,H\text{-}3}$ increased by <0.4 Hz for both anomers, but this cisoidal coupling is expected to be less sensitive to conformational change[13b]. The value of $^3J_{H\text{-}3,H\text{-}4}$ decreased from 4.8 Hz to 3.2 Hz in **3a**, and from 6.3 Hz to 5.3 Hz in **3b**; these changes, however,

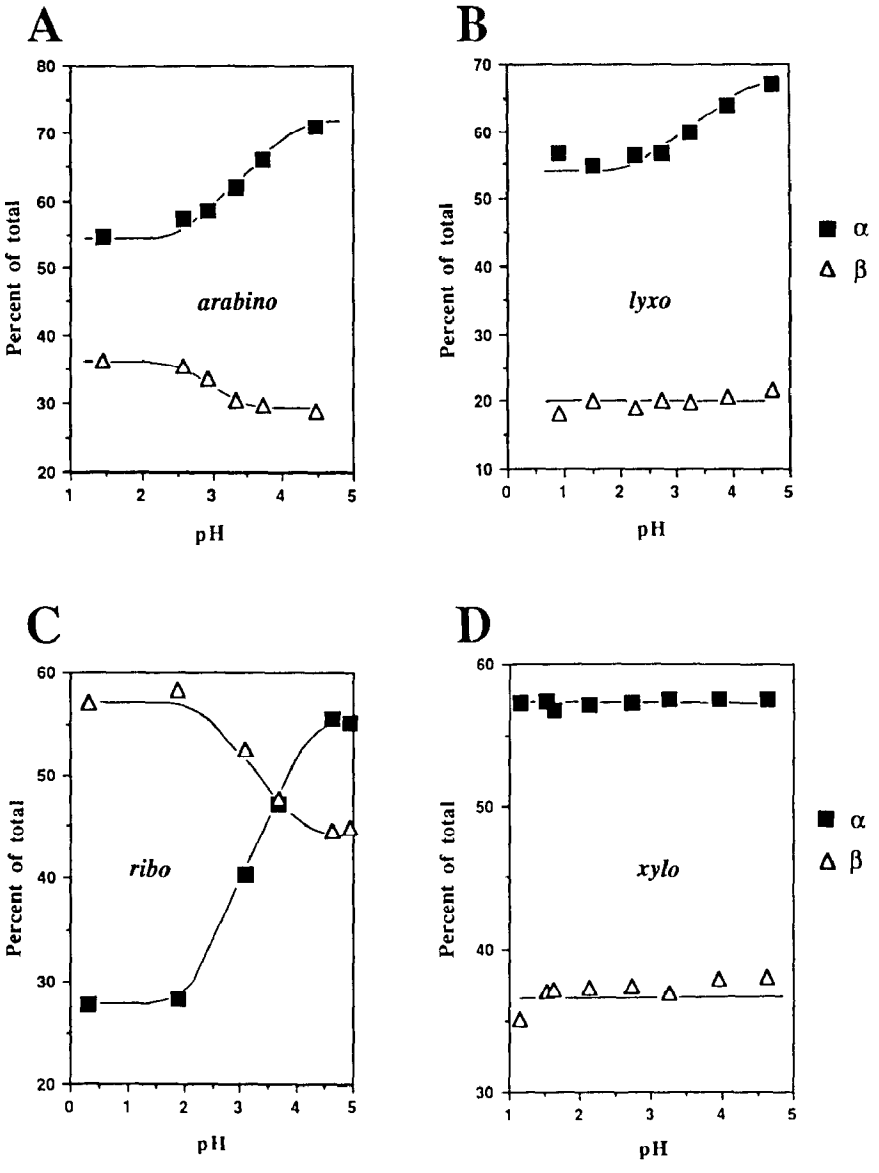


Fig. 6. The effect of solution pH on the percent of α- and β-furanose forms of the penturonic acids **1–4** in aqueous solution (0.2M in 30% ^2H_2O at 22°).

may be partly due to alteration of the Karplus curves caused by COOH ionization. In contrast, the relative proportions of **4a** and **4b** are unaffected by solution pH, and ^{1}H chemical shift titrations conducted on **4** showed little (< 0.5 Hz) change in $^3J_{H-1,H-2}$, $^3J_{H-2,H-3}$, and $^3J_{H-3,H-4}$ between pH 1.4 and 4.7 for both anomers. Hence, these data suggest that the ring conformations of **3a** and **3b** may depend on the state of ionization of the COOH group, and that conformational factors may contribute to the observed dependence of anomeric distribution on solution pH.

Isomerization of D-*riburonic acid.* — D-(1-^{13}C)Riburonic acid (**3**) isomerized to the corresponding (1-^{13}C)-4-pentulosonic acid (**6**) at pH 5.0 and 50° (Fig. 7). The fates of the enriched C-1 carbons in **3** were monitored during the reaction, and the appearance of two new signals due to enriched carbons at 67.8 and 67.4 p.p.m. after 48 h of reaction in a mixture of H_2O and ^2H_2O indicated the presence of **6** and its 1-^{13}C, 1-^{2}H-substituted derivative (Fig. 7A,B; Scheme 2). The latter compound was generated by solvent deuterium exchange. Although probably present in the final mixture, the 1-^{13}C, 1,1-$^2H_2$-substituted compound would not be easily detected due to increased ^{13}C–^{2}H splitting and loss of nuclear Overhauser enhancement (n.O.e.). A similar experiment conducted with D-(2-^{13}C)riburonic acid produced a new enriched-carbon signal at 212 p.p.m. (Fig. 7C), which is consistent with the expected C-2 resonance of **6** in its acyclic (*keto*) form. By analogy to the 2-pentuloses[10], little hydrated form is expected in aqueous solutions of **6**.

The conditions used to isomerize **3** to **6** are mild compared to those required for the isomerization of D-ribose to D-ribulose. Indeed, in the latter case, alkaline treatment (> pH 10) is required, and the yield of ribulose is low owing to its rapid rate of degradation in aqueous base.

D-Arabinuronic acid (**1**) and D-xyluronic acid (**4**) also isomerize under similar solution conditions to give 2-keto compounds, but no 2-keto product was formed from D-lyxuronic acid (**2**).

Structural interpretation of spin-couplings in **1–5.** — The values of $^1J_{C-1,C-2}$ in **1–4** (Fig. 5B, Table V) depend on anomeric configuration, with anomers having O-1,O-2-*cis* giving smaller couplings (42.6 ± 1.4 Hz), in both their free acid and ionized states, than anomers having these atoms *trans* (46.1 ± 0.5 Hz). This dependence on anomeric

TABLE VIII

Values of the ^{1}H–^{1}H coupling constants for 2-deoxy-D-*erythro*-penturonic acid in ^2H_2O[a]

Compound	*Coupled nuclei*					
	*H-1,H-2*R	*H-1,H-2*S	*H-2*R,*H-2*S	*H-2*R,*H-3*	*H-2*S,*H-3*	*H-3,H-4*
α-Furanose (**5a**)	2.0 (2.4)	5.6 (5.5)	−14.3 (−14.2)	2.0 (3.7)	6.6 (7.1)	3.1 (4.5)
β-Furanose (**5b**)	4.2 (4.0)	5.2 (5.1)	−13.9 (−14.0)	6.2 (6.5)	5.0 (5.7)	3.5 (4.4)
Methyl β-glycoside	4.5	4.5		5.5	5.5	3.6

[a] Measured on compounds in their free acid states (pH 1.6), reported in Hz, accurate to ± 0.1 Hz. Values in parentheses are couplings in the corresponding 5-*O*-methyl-2-deoxy-D-*erythro*-furanoses.

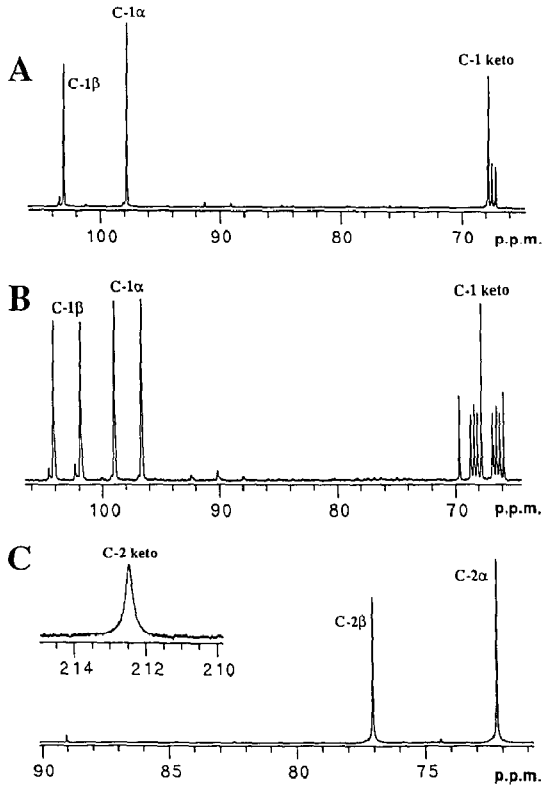
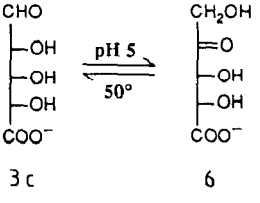


Fig. 7. *A*, The ^{1}H-decoupled ^{13}C-n.m.r. spectrum of D-(1-^{13}C)riburonate after incubation at pH 5.0 and 50° in $H_2O/^{2}H_2O$, showing the formation of 1-^{13}C-substituted and 1-^{13}C; 1-^{2}H-substituted *keto* forms having C-1 chemical shifts of 67.8 p.p.m. and 67.4 p.p.m., respectively. The identity of the 1-^{13}C; 1-^{2}H-substituted *keto* form was supported by the observed one-bond ^{13}C–^{2}H coupling (22.1 Hz) and the 0.4 p.p.m. upfield isotope shift of C-1 upon deuteration; *B*, the ^{1}H-coupled ^{13}C-n.m.r. spectrum obtained on the same sample as in *A*, showing a triplet for the C-1 signal (CH_2OH) of the nondeuterated *keto* form, and a doublet of triplets for the C-1 signal of the singly deuterated *keto* form ($CH^{2}HOH$); *C*, the ^{1}H-decoupled ^{13}C-n.m.r. spectrum of D-(2-^{13}C)riburonate after incubation at pH 5.0 and 50°.



Scheme 2

TABLE IX

Values of the ^{13}C–^{1}H coupling constants for the D-penturonic acids in $^{2}H_2O$[a]

Compound	*Coupled nuclei*						
	C-1,H-1	*C-2,H-2*	*C-3,H-3*	*C-4,H-4*	*C-1,H-2*	*C-1,H-3*	*C-1,H-4*
α-D-Arabinuronic acid (**1a**)	173.4	151.6	152.8	153.1	br.	br.	1.8
β-D-Arabinuronic acid (**1b**)	174.3	149.0	150.5	152.0	br.	1.5	3.6
2-Deoxy-α-D-*erythro*-penturonic acid (**5a**)	174.3	133.6[b]	153.9	154.0			
2-Deoxy-β-D-*erythro*-penturonic acid (**5b**)	173.7	133.4[b]	153.9	153.2			
α-D-Lyxuronic acid (**2a**)	171.7	146.5	157.3	150.9	4.9	5.0	
β-D-Lyxuronic acid (**2b**)	176.4	147.1	158.2	152.3	0		
α-D-Riburonic acid (**3a**)	174.1	151.2	154.6	154.9	br.		
β-D-Riburonic acid (**3b**)	174.2	153.9	150.9	154.0	0		3.2
α-D-Xyluronic acid (**4a**)	174.0	152.6	155.2	153.2	3.3	3.6	0
β-D-Xyluronic acid (**4b**)	174.1	155.5	156.9	151.8	br.	br.	0

[a] Measured on compounds in their free acid states (pH 1.6), reported in Hz, accurate to ± 0.1 Hz; "br." denotes broadened signal. [b] For **5a** and **5b**, $^{3}J_{C-1,H-2R} = {}^{3}J_{C-2,H-2S}$.

configuration has been noted previously in other aldofuranoses[2,13b] and ketofuranoses[19].

The values of $^1J_{C,H}$ in **1–4** (Table IX) depend on the carbon site under consideration, with $^1J_{C\text{-}1,H\text{-}1}$ (174.0 ± 1.3 Hz) larger than $^1J_{C\text{-}2,H\text{-}2}$ (150.9 ± 3.2 Hz), $^1J_{C\text{-}3,H\text{-}3}$ (154.6 ± 2.9 Hz), and $^1J_{C\text{-}4,H\text{-}4}$ (152.8 ± 1.3 Hz). A similar trend was noted previously in the aldotetrofuranoses[20].

It is well known that furanose rings are conformationally flexible structures, and thus the interpretation of physical parameters obtained on these rings in solution (*e.g.*, chemical shifts and spin-coupling constants) is complicated by conformational averaging[21]. Hence, conformational assignments are not made in the following discussion, but ^1H–^1H coupling behavior in **1–4** (Table V) is compared to that observed in the corresponding 5-*O*-methylpentoses[2]. This comparison allows a reasonable assessment of the effect on ring conformation of converting an exocyclic group at C-4 of the furanose ring from $-CH_2OCH_3$ to -COOH.

Because of the known influences of substituents on the shape and amplitudes of Karplus curves for $^3J_{H,H}$, direct comparisons of $^3J_{H\text{-}1,H\text{-}2}$ and $^3J_{H\text{-}2,H\text{-}3}$ only are allowable between the two series of compounds, as these couplings describe endocyclic torsions for fragments well removed from the site of substitution. An inspection of these couplings (Tables V and VIII) shows similar values ($^3J_{H,H}$ differing by <0.5 Hz) for **2b, 3a, 3b,** and **4b,** and their corresponding 5-*O*-methylpentoses, suggesting that, in these configurations, conversion to the uronic acid (free acid) does not affect ring conformation significantly. In contrast, notable differences are observed for the *α-arabino* (**1a**), *β-arabino* (**1b**), *α-lyxo* (**2a**), and *α-xylo* (**4a**) configurations. For example, in **1a**, $^3J_{H\text{-}1,H\text{-}2}$ (1.8 Hz) and $^3J_{H\text{-}2,H\text{-}3}$ (2.9 Hz) are significantly smaller than corresponding couplings in 5-*O*-methyl-α-D-arabinofuranose (2.7 and 4.6 Hz, respectively), suggesting a greater predominance in the former of south (*e.g.*, E_1) conformers in which the C-1–O-1 bond is quasiaxial. Thus, the conformational response of furanose rings to the conversion from a $-CH_2OCH_3$ to a -COOH substituent at C-4 depends on ring configuration.

It is interesting to note that a comparison of corresponding ^1H–^1H couplings in methyl pentofuranosides[13b], penturonic acids (free acids), and 5-*O*-methylpentoses[2] shows similar values for the *β-lyxo*, *α-ribo*, *β-ribo*, and *β-xylo* configurations. Thus, these configurations, in addition to being relatively insensitive to substituent changes at C-4 (see above), appear to be relatively tolerant of substituent changes at O-1, when compared to **1a, 1b, 2a**, and **4a**. This observation suggests that strong internal factors control ring conformation in the methyl glycosides and reducing furanoses having the *β-lyxo*, *α-ribo*, *β-ribo*, and *β-xylo* configurations, and these factors may function to dampen the effect of C-4 and O-1 substituent structure on ring conformation.

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