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DL-APIOSE SUBSTITUTED WITH STABLE ISOTOPES: SYNTHESIS, N.M.R.-SPECTRAL ANALYSIS, AND FURANOSE ANOMERIZATION

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ABSTRACT

The branched-chain pentose DL-apiose has been synthesized in good yield by a new and simple chemical method that can be adapted to prepare (1-^{13}C)-, (2-^{13}C)-, (1-^{2}H)- and/or (2-^{2}H)-enriched derivatives. N.m.r. spectra (^{1}H- and ^{13}C-) have been interpreted with the aid of selective (^{13}C)- and (^{2}H)-enrichment, and 2D and ^{13}C{^{13}C}-n.m.r. spectra. The solution composition of DL-(1-^{13}C)apiose in $^{2}H_2O$, determined by ^{13}C-n.m.r. spectroscopy, has been found to differ from that determined previously by ^{1}H-n.m.r. spectroscopy. Several ^{13}C–^{1}H and ^{13}C–^{13}C couplings have been measured and interpreted in terms of apiofuranose ring conformation. Ring-opening rate-constants of the four apiofuranoses [3-*C*-(hydroxymethyl)-α- and -β-D-erythrofuranose, and 3-*C*-(hydroxymethyl)-α- and -β-L-threofuranose] have been determined by ^{13}C-saturation-transfer n.m.r. spectroscopy, and compared to those obtained previously for the structurally related tetrofuranoses.

INTRODUCTION

The branched-chain aldose D-apiose [3-*C*-(hydroxymethyl)-D-*glycero*-aldotetrose] plays an integral role in the biochemistry of plants[1], occurring in the form of UDP-D-apiose or as a glycoside attached to other cell components. The solution behavior of this pentose is characterized by its ability to form four different furanoses in solution, as described by Angyal[2–4] using ^{1}H-n.m.r. spectroscopy. Two of these forms (**1** and **2**) have the D configuration, and two (**3** and **4**) have the L configuration; apparently, 3-*C*-(hydroxymethyl)-α-D-erythrofuranose (**1**) is the biologically important structure[1,5], being attached in α linkage to UDP. In addition, two linear forms are theoretically possible, namely, aldehyde **5** and its hydrate **6** (1,1-*gem*-diol, in aqueous solution only), but their detection in solution has not been reported. In general, linear forms of aldoses are found in small proportions (<2 mol-%) in solution, and their detection by ^{13}C-n.m.r. spectroscopy often requires use of [^{13}C]-enriched compounds[6–8].

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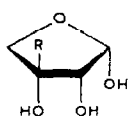

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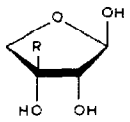

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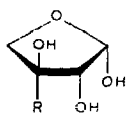

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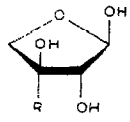

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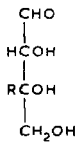

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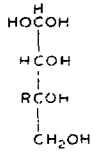

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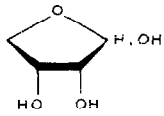

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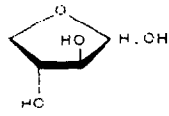

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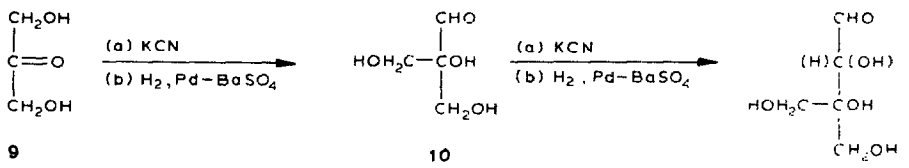
Because of its ability to form rings having *erythro* and *threo* configurations, apiose is an ideal paradigm by which to study the effects of furanose-ring configuration on rates of ring-opening and -closing[6,8]. In addition, an apiofuranose can be viewed as a derivative of a tetrofuranose (**7** or **8**), useful in evaluating the effect of $-CH_2OH$ substitution on anomerization rates. Sugars enriched with ^{13}C at the anomeric carbon atom facilitate ^{13}C-n.m.r. studies of anomerization[6,8], and the availability of [^{13}C]- or [^{2}H]-enriched apiose, or both, may promote studies of its metabolism in plants. For these reasons, we investigated development of a convenient method by which to prepare derivatives enriched with stable isotopes.

This report describes a new and simple method by which to synthesize DL-apiose. In contrast to other methods[9–13], the procedure is short, and allows easy incorporation of ^{13}C at C-1 or C-2, or both, and of ^{2}H at H-1 or H-2 or both. An inexpensive and readily available, achiral starting-material, namely, 1,3-dihydroxy-2-propanone (dihydroxyacetone) **9**, is used to synthesize the target sugar in good yield by applying two consecutive cyanohydrin and reduction cycles[14–16] (see Scheme 1). With the aid of selective [^{13}C]-enrichment, 2D n.m.r., $^{13}C\{^{13}C\}$ spectra, and selective [^{2}H]-labeling, DL-apiose (**11**) in $^{2}H_2O$ has been characterized by ^{1}H- and ^{13}C-n.m.r. spectroscopy. Solution composition in $^{2}H_2O$ has been re-evaluated from the ^{13}C spectra of the [1-^{13}C]-enriched derivative. The furanose conformation has been addressed with the use of ^{13}C–^{1}H and ^{13}C–^{13}C spin-couplings, and, for the four apiofuranoses ring-opening rate constants, obtained by ^{13}C saturation-transfer n.m.r. spectroscopy[6,8,17,18], are discussed.



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Scheme 1

EXPERIMENTAL

Materials. — 1,3-Dihydroxy-2-propanone (dihydroxyacetone) and palladium–barium sulfate (Pd–$BaSO_4$, 5%) were purchased from Sigma Chemical Co. Potassium (^{13}C)cyanide ($K^{13}CN$, 99 atom-% of ^{13}C) and deuterium oxide (^2H_2O, 99.8 atom-% of ^{2}H) were purchased from Cambridge Isotope Laboratories. Deuterium gas ($^2H_2$, 98 atom-% of ^{2}H) was purchased from Matheson Gas Products. 1,2:3,3^1-Di-*O*-isopropylidene-D-3-*C*-(hydroxymethyl)-α-D-erythrofuranose was purchased from Pfanstiehl Laboratories, Inc., and converted into D-apiose by mild hydrolysis with acid. Standard iodine solutions (0.1M) used for sugar quantitation by hypoiodite oxidation were purchased from Fisher Scientific Company. Anhydrous sodium thiosulfate was purchased from Aldrich Chemical Co.

Instrumentation. — N.m.r. spectra (^{1}H-, 300 MHz, and ^{13}C-, 75 MHz) were recorded with a Nicolet NT-300 F.t.-NMR spectrometer equipped with quadrature-phase detection and a 293B pulse programmer. Heteronuclear *J*-spectroscopy[19] and ^{13}C–^{1}H shift-correlation spectroscopy[20] were conducted with the same spectrometer, using software supplied by GE n.m.r. Systems. ^{13}C-saturation-transfer spectroscopy was conducted at 75 MHz, using the NT-300 hardware modifications and pulse program described previously[21a]. Selective ^{13}C-decoupled, ^{13}C-n.m.r. spectra (^{13}C{^{13}C} spectra) were obtained on the NT-300 n.m.r. instrument by using a broadband decoupling accessory (F3 Decoupler Unit) supplied by GE NMR Systems.

The mass spectrum of the peracetylated alditol acetate derivative of DL-apiose was recorded with a Finnigan Mat mass spectrometer, Model 8430, operated in the positive-ion, chemical-ionization mode with isobutane and with a source temperature of 140° (direct probe). The peracetylated alditol acetate derivative was prepared as described by Blakeney *et al.*[21b].

Reaction of dihydroxyacetone (**9**) *with cyanide.* — To an aqueous solution of KCN (16.3 g, 250 mmol) in H_2O (100 mL), adjusted to pH 7.5 with conc. HOAc at 23°, was added a solution of dihydroxyacetone (**9**; 4.5 g, 50 mmol) in H_2O (5 mL) with stirring in a two-necked flask equipped with a pH electrode, and the procedure was performed in a well-vented hood (to avoid exposure of the operator to HCN vapors). The pH of the mixture was maintained at 7.5 by dropwise addition of dilute HOAc or NaOH, as needed. (The vessel need not be air-tight, as HCN is very soluble in water at 23°, and minimal loss to the atmosphere occurs. However, during the initial adjustment of the pH of the KCN solution with conc. HOAc, considerable heat is generated, and cooling of the vessel in an ice-bath is advised in order to prevent escape of HCN.)

After 10 min, the mixture was assayed by g.l.c. as described previously[22]; unreacted **9** was eluted first, followed by the product cyanohydrin. The solution, adjusted to pH 4.3, was aerated for 3 h with N_2 in a well-vented hood to remove the excess[14] of HCN; failure to remove the excess of HCN from the reaction mixture will result in poisoning of the catalyst in the next step, causing complete

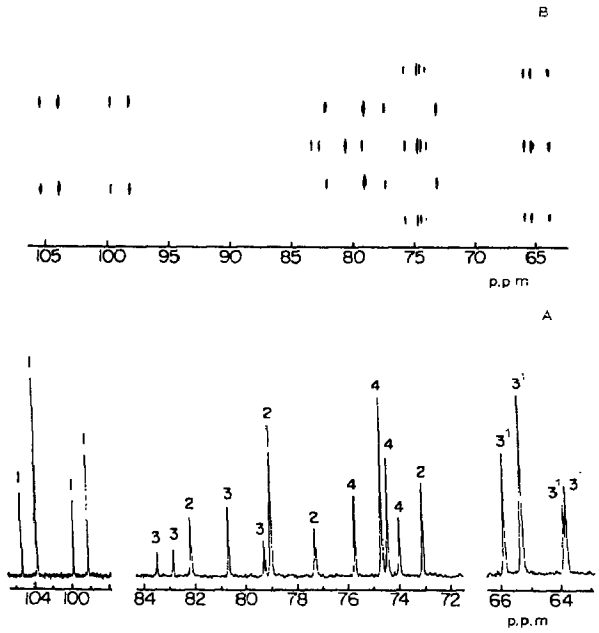

Fig. 1. (A) The ^{1}H-decoupled, ^{13}C-n.m.r. spectrum (75 MHz) of DL-apiose in ^2H_2O, showing the presence of four major furanose forms. Assignments of C-1, C-2, C-3, C-3^1, and C-4 are shown. (B) A 2D, heteronuclear *J* spectrum of DL-apiose, showing multiplets due to one-bond, ^{13}C–^{1}H coupling in the second dimension. Assignments of unprotonated (C-3), singly protonated (C-1, C-2) and doubly protonated (C-3^1, C-4) atoms in (A) were made, based on this *J* spectrum.

inhibition of reduction of the nitrile. The pH of the solution was readjusted to 4.3 with conc. HOAc and to pH 1.7 with dilute H_2SO_4, and the nitrile was reduced at atmospheric pressure with pre-reduced 5% Pd–$BaSO_4$ (3.0 g) and H_2 until the uptake of gas was complete (~6 h). The suspension was filtered with suction to remove spent catalyst, the solution was treated batchwise with $BaCO_3$ to remove SO_4^{2-}, the suspension filtered, and the filtrate deionized by batchwise treatment with an excess of Dowex-1 (HCO_3^-) and Dowex-50 (H^+) resins. The solution was concentrated to 50 mL at 30° *in vacuo*, and the concentrate chromatographed on a column (4.4 × 110 cm) of Dowex-50 X-8 (Ca^{2+}) resin[23] (200–400 mesh) using distilled water as the eluant (1 mL/min). Fractions (18 mL) were assayed for aldose with phenol–sulfuric acid[24], and those containing **10** (Nos. 54–72) were pooled, and concentrated at 30° *in vacuo* to ~30 mL (50% yield, based on **9**, KCN–$AgNO_3$ assay[22]). The ^{13}C-n.m.r. spectrum of **10** in ^2H_2O showed the presence of a monomer–oligomer equilibrium similar to that observed for DL-glyceraldehyde[13]; in dilute solution, a characteristic signal at 92.4 p.p.m. was observed and, by analogy to DL-glyceraldehyde, assigned to C-1 of the monomeric hydrate (1,1-*gem*-diol) form of **10**.

DL-*Apiose* [*3-C-(hydroxymethyl)*-DL-glycero-*aldotetrose*] (**11**). — Compound **10** was treated with one equivalent of KCN under the conditions described for **9**.

After assay by g.l.c.[22] as already described, the compound was reduced, and the solution de-ionized and chromatographed as already described; DL-apiose was eluted in fractions 84–96, in 50% yield based on **10** ($NaIO_3$ assay[25]). After evaporation to a syrup *in vacuo* at 30°, the product (DL-apiose) was characterized by comparison of its ^1H- and ^{13}C-n.m.r. spectra (see Fig. 1A) in ^2H_2O with those of D-apiose generated by acid hydrolysis of the di-*O*-isopropylidene derivative (obtained commercially); the spectra of the product DL-apiose and of the authentic D-apiose were identical. The mass spectrum of the peracetylated alditol derivative of product DL-apiose contained a predominant fragment at *m/z* 303, corresponding to the loss of a single OAc group from the structure. The quasi-molecular ion $(M + 1)^+$ at *m/z* 363 was not observed under the experimental conditions used (see Instrumentation section).

Preparation of isotopically substituted DL-*apiose.* — DL-(1-^{13}C)Apiose and DL-(2-^2H)apiose were prepared by using the foregoing protocols, substituting $K^{13}CN$ and $^2H_2$ gas, respectively, in the cyanide reaction on **10**, and in the catalytic reduction of 2-C-(hydroxymethyl)glyceronitrile to give **10**. Reactions were carried out with 10 mmol each of **10** and $K^{13}CN$, to prepare DL-(1-^{13}C)apiose. DL-(2-^2H)Apiose was prepared by starting with 20 mmol of **9**; the cyanide reaction on **9**, and the subsequent catalytic reduction with Pd–$BaSO_4$ and $^2H_2$, were performed in ^2H_2O as described in previous reports[26,27]. Yields at each step were similar to those reported on using isotopically normal reactants.

RESULTS AND DISCUSSION

Synthesis of labeled DL-*apiose.* — The synthetic route shown in Scheme 1 for the preparation of DL-apiose is shorter and easier than those described previously[9–13], gives comparable yields, and is readily adapted to preparing derivatives substituted with stable isotopes. Other aldehydes and ketones (*e.g.*, 3-hydroxy-2,2-dimethylpropanal, acetol) can be substituted for **9** and **10**, in order to generate other C-3-substituted furanoses. Although not as attractive as the pure D isomer, the product DL mixture may be suitable for some studies of plant metabolism.

A five-fold excess of cyanide is needed in order to achieve high (>90%) conversion of dihydroxyacetone (**9**) into 2-*C*-(hydroxymethyl)glyceronitrile. In contrast, glyceraldehyde is converted into tetrononitriles in comparable yield with only one molar equivalent[14,15]. The conversion of **10** into its corresponding cyanohydrin is also effected in >90% yield with one equivalent of cyanide. The aldononitriles are reduced to aldoses with H_2 and Pd–$BaSO_4$ at pH 1.7 in 60–70% yield. (1-^{13}C)-, (2-^{13}C)-, (1-^2H)-, and (2-^2H)-Substituted DL-apiose can be prepared from $K^{13}CN$ or $^2H_2$ as described for other aldoses[14–16,26,27]. In this study, DL-(1-^{13}C)apiose and DL-(2-^2H)apiose were prepared in order to facilitate ^{13}C- and ^1H-n.m.r. studies (see later).

DL-(1-^{13}C)Apiose (99 atom-% of ^{13}C) reacts with sodium molybdate[28,29] to generate a mixture of (1-^{13}C)- and (2-^{13}C)-substituted compounds (data not shown),

TABLE I

^{13}C CHEMICAL SHIFTS FOR APIOFURANOSES IN ^2H_2O

Compound	*Chemical shift (p.p.m.)*[a]				
	C-1	*C-2*	*C-3*	*C-3'*	*C-4*
1	97.9	73.0	79.1	65.8	74.4
2	103.4	78.9	80.5	65.2	74.6
4	105.0	82.0	82.7	63.76	75.7
3	99.5	77.2	83.3	63.84	73.9

[a]Values are accurate to within ±0.1 p.p.m. and are referenced (external) to the anomeric-carbon signal of β-D-(1-^{13}C)glucopyranose (97.4 p.p.m.)

but also produced were labeled by-products whose identities were not determined. At equilibrium, this reaction should yield a 1:1 mixture of (1-^{13}C)- and (2-^{13}C)-apiose, each containing ~49 atom-% of ^{13}C. Apparently the presence of a hydroxymethyl substituent on C-3 does not prevent molybdate-catalyzed epimerization of aldoses, provided that OH groups are present[29] at C-2 and C-3.

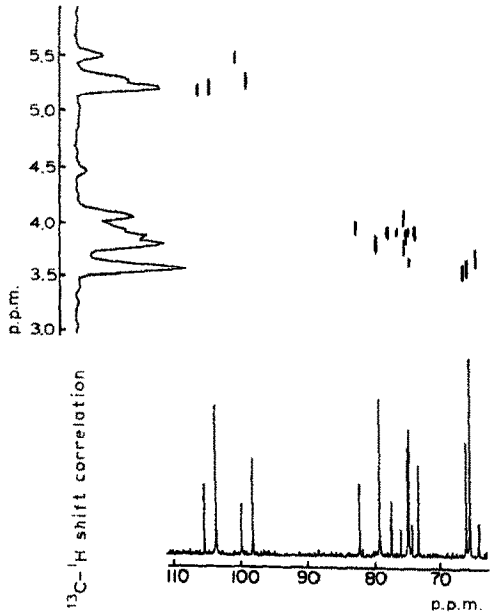

Fig. 2. A 2D, ^{13}C–^1H chemical-shift correlation map of DL-apiose. The anomeric carbon atoms (98–105 p.p.m.) are easily correlated with anomeric protons (5.2–5.6 p.p.m.) (see Fig. 3A for the high-resolution, 300-MHz, ^1H-n.m.r. spectrum of the anomeric region) and assignments can be made with confidence. Double cross-peaks are observed for C-4 of **2** and **3**, illustrating the nonequivalence of the attached, diastereotopic protons, and facilitating their assignment in the high-resolution, ^1H-n.m.r. spectrum (Fig. 3B). In general, inspection of this map permitted the location of specific protons of each tautomer in the high-resolution, ^1H-n.m.r. spectrum (Fig. 3) *via* correlation with an assigned ^{13}C-n.m.r. spectrum.

TABLE II

^1H CHEMICAL SHIFTS FOR APIOFURANOSES IN ^2H_2O

Compound	*Chemical shift (p.p.m.)*[a]				
	H-1	*H-2*	*H-3',H-3"(exo)*	*H-4(endo)*	*H-4'(endo)*
1	5.34	~3.99	3.62	n.d.	n.d.
2	5.28	3.88	3.65	4.13	3.85
4	5.26	4.03	3.81[b], 3.70[c]	n.d.	n.d.
3	5.56	~3.99	3.83[b], 3.69[c]	n.d.	3.67

[a]Relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate, and accurate to within ±0.01 p.p.m. [b,c]Assignments may have to be reversed. The entry "n.d." designates values which were not determined exactly but that lie between 3.94 and 4.02 p.p.m. (see Fig. 3).

^1H- and ^{13}C-N.m.r.-spectral characterization. — The elucidation of the fundamental solution properties of a reducing sugar involves (*a*) the identification of specific tautomers and their relative abundances (thermodynamics)[4,30], (*b*) an evaluation of the conformational preferences–dynamics of each tautomer[30–33a], and (*c*) an understanding of the kinetics of tautomer interconversion[6,8,21a,34–36]. These features can be probed by interpreting the static (*i.e.* chemical shifts, spin–spin couplings) and dynamic (*i.e.* nuclear relaxation and Overhauser effects, line-broadening effects, saturation–inversion transfer) n.m.r. parameters of the system, as illustrated for apiose.

The composition of aqueous (^2H_2O) solutions of D-apiose at 31° determined[4,37] by ^1H-n.m.r. spectroscopy at 100 MHz has been reported as follows: 3-*C*-(hydroxymethyl)-α-D-erythrofuranose (**1**), 22%; 3-*C*-(hydroxymethyl)-β-D-erythrofuranose (**2**), 54%; 3-*C*-(hydroxymethyl)-α-L-threofuranose (**4**), 9%; and 3-*C*-(hydroxymethyl)-β-L-threofuranose (**3**), 15%. The linear forms **5** and **6** were not observed. Because of the close proximity of the H-1 resonances of **2** and **4** at 100 MHz, some uncertainty in these proportions might be expected. In particular, the relative abundances of **3** and **4** are somewhat surprising, based on previous observations of the effect of furanose structure on anomeric proportions[7]. In contrast to ^1H-n.m.r.-spectral results, **4** is predicted to be *more stable* than **3**, because of its more favorable O-1–O-2-*trans* configuration.

The ^{13}C-n.m.r. spectrum of DL-apiose in ^2H_2O is shown in Fig. 1A. The assignment of the anomeric-carbon signals (see Table I) was made by 2D ^{13}C–^1H shift-correlation spectroscopy (see Fig. 2), using the H-1 assignments made previously[3,37] (see Table II). The ^{13}C-n.m.r. spectrum of DL-(1-^{13}C)apiose contains the C-1 signal of the hydrate **6** (90.8 p.p.m.), but, at 25°, the C-1 signal of the aldehyde **5** could not be detected in the 200–210 p.p.m. region of the spectrum where this signal is expected[4,6–8,21a] (this problem is discussed in more detail later). At 25°, the solution composition of DL-(1-^{13}C)apiose was found to be: **1**, 26; **2**, 44; **4**, 16; **3**, 14; and **6**, ~0.1%. In contrast to previous ^1H-n.m.r. studies[4,37], the ^{13}C results show that **4** is more preponderant than **3**, although the difference is small. In general, the

proportions of forms determined by ^{13}C-n.m.r. spectroscopy differ significantly from those reported from ^{1}H-n.m.r. studies. The bulky 3-hydroxymethyl group of apiose is *trans* to the 2-hydroxyl group in the *erythro* configuration (**1, 2**) and presumably accounts for the preponderance of these isomers (7:3 *erythro:threo*). The β-D-*erythro* anomer **2** is more stable than the corresponding α anomer (**1**) due to the more favorable O-1–O-2-*trans* configuration in the former; a similar argument can be invoked to explain the slight preference of the α-L-*threo* (**4**) over the β-L-*threo* (**3**) configuration.

Aqueous solutions of apiose at 25° contain significantly less aldehyde (≤0.01%) and hydrate (~0.1%) than those of the tetroses (**7** and **8**; ~2 and 10%, respectively)[6]. This difference may be attributed, in part, to entropic effects and to the Thorpe–Ingold effect[33b,c]. The presence of two primary hydroxyl groups in **11**, as opposed to only one in the tetroses, increases the probability of ring closure and lowers the equilibrium percentage of aldehyde. The Thorpe–Ingold effect[33b,c] predicts that substitution will encourage cyclization, and is manifested in enhanced reaction-rates (*i.e.*, ring-closure) and solution thermodynamics. The lessened carbonyl content is reflected in the smaller percentage of hydrate (~10% for tetroses[6], ~0.1% for **11**); for simple, unsubstituted aldoses, the hydrate:aldehyde ratio in aqueous solution appears[4] to be rather constant at ~8:1.

The sixteen nonanomeric ^{13}C signals (see Fig. 1A) were assigned by obtaining a 2D heteronuclear *J*-resolved spectrum, to determine the proton multiplicity of each carbon resonance (see Fig. 1B). Resonances at 82.0, 78.9, 77.2, and 73.0 p.p.m. in the 1D ^{13}C spectrum appear as doublets in the *J*-resolved experiment, and were therefore assigned to the singly protonated carbon atoms (C-2) [confirmed from the 1D ^{13}C spectrum of DL-(1-^{13}C)apiose, in which these signals are split by one-bond, ^{13}C–^{13}C coupling], whereas unprotonated carbon atoms (C-3) (not split in the *J*-spectrum) resonate at 83.3, 82.7, 80.5, and 79.1 p.p.m. Hydroxymethyl carbon signals appear as triplets in the *J*-resolved spectrum (see Fig. 1B), with the exocyclic (C-3[1]) carbon atoms resonating upfield from the endocyclic (C-4) carbon atoms, as expected from effects of *O*-substitution on ^{13}C chemical-shifts[38]. These data were used in conjunction with relative intensities to assign the ^{13}C spectrum tentatively (see Table I).

To confirm the assignments of C-2 (and H-2), the ^{1}H-n.m.r. spectrum of DL-(2-^{2}H)apiose was compared to that of the unenriched compound, in order to locate and assign the H-2 signals to specific tautomers on the basis of $^{3}J_{H1,H2}$ values (see Fig. 3, A and B). Using 2D ^{13}C–^{1}H shift-correlation spectroscopy (see Fig. 2), C-2 assignments *to each furanose anomer* could be made unambiguously (see Table I). These assignments are also confirmed by $^{1}J_{C\text{-}1,C\text{-}2}$ values (see Table III), which are useful in order to distinguish[7,39] furanose anomers having O-1–O-2-*trans* (**2, 4**) from those having O-1–O-2-*cis* (**1, 3**).

The unprotonated C-3 resonances of **1** and **2** were assigned (see Table I) from relative intensities. A similar approach, however, could not be used with confidence to assign the C-3 resonances of **3** and **4**, whose intensities are similar (see

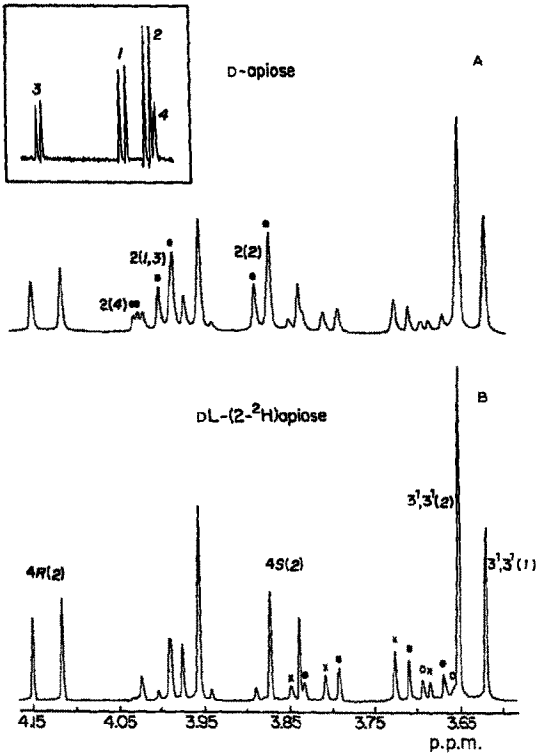

Fig. 3. (A) The high-resolution, ^{1}H-n.m.r. spectrum (300 MHz) of authentic D-apiose in ^2H_2O. The inset shows the anomeric region: downfield to upfield, H-1 of **3**, **1**, **2**, and **4**, respectively. Assignments of the H-2 resonances are shown; doublets for H-2 of **1** and **3** overlap, as suggested by the 2D correlation map (Fig. 2). The upfield half of the H-2 doublet of **2** overlaps with the downfield half of the H-4*S* doublet of **2**. (B) The high-resolution, ^{1}H-n.m.r. spectrum (300 MHz) of DL-(2-^{2}H)apiose in ^2H_2O, showing significant loss in the intensities of the H-2 signals, and verifying their assignments. The C-4 protons of **2** are nonequivalent (the *R*, *S* assignments are made by analogy to those made previously on β-D-erythrofuranose[45]), while the C-3^1 protons of **1** and **2** are equivalent. Resonances marked with (×) and (●) are the nonequivalent C-3^1 protons of **3** and **4** (see Table II). The doublet marked with (○) is one of the C-4 protons of **3**; the other C-4 proton resonates between 3.94 and 4.04 p.p.m., as indicated by the 2D correlation map (Fig. 2). Signals due to the C-4 protons of **1** and **4** overlap in the 3.94–4.01-p.p.m. region of the spectrum.

Fig. 1A). In these cases, assignments were made by selectively irradiating the C-1 resonances of (1-^{13}C)-substituted **3** and **4** and observing the selective collapse of the C-3 signals from doublets (caused by $J_{C\text{-}1,C\text{-}3}$) to singlets. As illustrated here, $^{13}C\{^{13}C\}$ spectra of (^{13}C)-substituted compounds can be very helpful when uncertainty exists in the assignment of signals of carbon atoms (especially unprotonated carbon atoms, where shift-correlation spectroscopy cannot be applied) *to specific tautomers*.

The assignments of the C-3^1 and 4-hydroxymethyl protons of **11** (see Table II) were made from complementary information obtained from heteronuclear *J* and shift-correlation spectroscopy (see Figs. 1 and 2), the magnitudes of $^2J_{H\text{-}H}$

TABLE III

^1H–^1H SPIN-COUPLING CONSTANTS[a] FOR APIOFURANOSES IN ^2H_2O

Compound	*Coupled nuclei*		
	H-1,H-2	*H-3',H-3"(exo)*	*H-4,H-4'(endo)*
1	4.8	e.q.	
2	4.6	e.q.	
4	1.4	~12.0	10.2
3	3.8	~12.0	

[a]Values are reported in Hz, and are accurate to within ±0.1 Hz. An entry "e.q." denotes nuclei that are magnetically equivalent at 300 MHz. No entry denotes couplings that could not be evaluated due to resonance overlap.

values (see Table III), and relative intensities. In contrast to hydroxymethyl protons in most sugars, the 3[1]-hydroxymethyl protons of **1** and **2** are magnetically equivalent, each set appearing as a single, relatively sharp resonance. In contrast, the 3[1] protons of **3** and **4** are nonequivalent (see Fig. 3B). Angyal *et al.*[37] observed similar behavior in methyl apiofuranosides. The structural factors responsible for this difference are unclear. The endocyclic 4-hydroxymethyl protons, at least for **2**, are magnetically nonequivalent (see Table II and Fig. 3B), like those of the tetroses and tetrofuranosides[7].

Apiofuranose ring conformation. — The interpretation of vicinal spin-couplings in furanose rings in terms of ring conformation is not straightforward, because of their inherent flexibility, even when multiple couplings are available[7]. The single, vicinal ^1H–^1H coupling ($^3J_{H\text{-}1,H\text{-}2}$) in apiofuranose rings provides insufficient information to define ring conformation, and additional information from ^{13}C–^1H and ^{13}C–^{13}C couplings may be useful. However, some insight may be obtained by comparing $^3J_{H\text{-}1,H\text{-}2}$ values (see Table III) with those found[7] in the structurally related tetrofuranoses **7** and **8**, which are as follows: α-threofuranose, 1.2 Hz;

TABLE IV

^{13}C–^{13}C SPIN-COUPLING CONSTANTS[a] FOR APIOFURANOSES IN ^2H_2O

Compound	*Coupled nuclei*			
	C-1,C-2	*C-1,C-3*	*C-1,C-3'*	*C-1,C-4*
1	43.9	br	2.5	—
2	47.7	3.3	2.8	—
4	46.0	2.7	~1.1	—
3	42.2	2.6	~1.5	—

[a]Values are reported in Hz, and are accurate to within ±0.1 Hz. A (—) entry means that no coupling was observed; "br" denotes broadening.

TABLE V

^{13}C–^{1}H SPIN-COUPLING CONSTANTS[a] FOR APIOFURANOSES IN $^{2}H_2O$

Compound	*Coupled nuclei*			
	C-1,H-1	*C-1,H-2*	*C-1,H-4(endo)*	*C-1,H-4'(endo)*
1	173.8			
2	170.4	~4.6	1.6	~4.4
4	172.3			
3	172.5			

[a]Values are reported in Hz, and are accurate to within ±0.1 Hz. No entry denotes couplings that were not determined.

TABLE VI

RING-OPENING RATE CONSTANTS[a] FOR APIOFURANOSES AND TETROFURANOSES

Compound	k_{open} *(±10%)*
1	0.096
2	0.066
4	0.090
3	0.41
α-D-Erythrofuranose[b]	0.40
β-D-Erythrofuranose[b]	0.19
α-D-Threofuranose[b]	0.11
β-D-Threofuranose[b]	0.36

[a]s^{-1}. Solution conditions: 0.3M aldose; 50mM acetate (Na^+) buffer; pD 5.0; 55°; in $^{2}H_2O$. [b]Values taken from ref. 6.

β-threofuranose, 4.1 Hz; α-erythrofuranose, 4.7 Hz; and β-erythrofuranose, 3.4 Hz. The conformational characteristics of the tetrofuranoses **7** and **8** and their methyl glycosides have been studied[7] by ^{1}H- and ^{13}C-n.m.r. spectroscopy, providing a basis for the following discussion.

The values of $^{3}J_{H\text{-}1,H\text{-}2}$ for **1** (4.8 Hz) and α-D-erythrofuranose (4.7 Hz) are similar, suggesting similar ring conformations (near E_1 on the pseudorotational itinerary)[7]. The magnitude[40] of $^{3}J_{C\text{-}1,C\text{-}3^1}$ (2.5 Hz; see Table IV) is consistent with a *quasi*-equatorial orientation of C-3[1] present in the E_1 (or ^{2}E) conformer. Furthermore, in E_1 (or ^{2}E), O-1 is *quasi*-axial, the preferred orientation due to the anomeric effect[41], which has been shown to be a major determinant of furanose ring conformation[7]. On the other hand, the $^{3}J_{H\text{-}1,H\text{-}2}$ values for **2** (4.6 Hz) and β-D-erythrofuranose (3.4 Hz) are significantly different. Angyal[42] argued from $^{3}J_{H\text{-}H}$ values that β-D-erythrofuranose exists mainly in two twist conformers ($^{3}T_2$ and $^{2}T_3$). The observed value of 2.8 Hz for $^{3}J_{C\text{-}1,C\text{-}3^1}$ of **2** suggests a favored *quasi*-equatorial orientation of C-3[1], which is found in the $^{2}T_3$ but not in the $^{3}T_2$ conformer. The

3[1]-hydroxymethyl group may make **2** less flexible than β-D-erythrofuranose; this decreased flexibility may be caused, in part, by the accommodation of a smaller C-2–C-3–C-4 endocyclic bond-angle in **2**, in response to a larger exocyclic C-3[1]–C-3–O-3 bond-angle (relative to the H-3–C-3–O-3 bond-angle in β-D-erythrofuranose) due to steric effects[33c]. In order to accommodate a *quasi*-equatorial C-3[1] in $^2T_3$, however, O-1 must be *quasi*-equatorially oriented, in opposition to the anomeric effect[41]. The differential coupling between C-1 and the C-4 protons (see Table V) is consistent with a favored conformation for **2** that is near $^2T_3$. This conformational assignment is approximate. Compound **2** may favor the ^oE, E_1, ^2E, or E_3 conformation, all of which are consistent with the coupling data available.

Values of $^3J_{H\text{-}1,H\text{-}2}$ for **3** and **4** are similar to the corresponding values for the D-threofuranoses **8**. The $^3J_{C\text{-}1,C\text{-}3^1}$ values for **3** and **4** (av. 1.3 Hz) are significantly smaller than those for **1** and **2** (av. 2.7 Hz), perhaps indicating a more *quasi*-axial orientation of C-3[1] in the *threo* isomers. These data suggest conformations near E^o and E_o for **3** and **4**, respectively. In all, the conformational conclusions drawn from the available couplings need further confirmation, but this will require a more extensive treatment of long-range ^{13}C–^1H and ^{13}C–^{13}C couplings in these rings, which are not easily obtained.

The apparently smaller $^2J_{HH}$ values (10.2 Hz) for endocyclic (*i.e.*, C-4) compared to $^2J_{HH}$ (12.0 Hz) for exocyclic (*i.e.*, C-3[1]) hydroxymethyl protons (see Table III) may indicate a larger H–C–H bond-angle in the former[43].

Apiofuranose anomerization. — As already discussed, the linear aldehyde form (**5**) of DL-(1-^{13}C)apiose in ^2H_2O could not be detected by ^{13}C-n.m.r. spectroscopy at 25°. This behavior differs markedly from that[6,7] of tetroses **7** and **8**, and is caused by the Thorpe–Ingold effect[33b,c] manifested both in thermodynamic equilibria and ring-closure rates. Not only is less of the carbonyl form predicted by this effect for **11**, but ring closing should be faster for **11** than for **7** and **8**, resulting, for the carbonyl carbon atom, in a broader signal that is more difficult to detect. At higher temperatures, the percentage of carbonyl form increases[4,6,8], thereby facilitating detection. However, raising the temperature also enhances the anomerization rates, and the resulting additional line-broadening of the aldehyde carbon resonance due to chemical exchange can nullify an increase in signal intensity. Therefore, a solution pH was chosen where these exchange rates are minimal[8] (pH ~4.5). At this pH, the aldehyde form **5** was observed in aqueous (^2H_2O) solutions of DL-(1-^{13}C)apiose ($\delta_{C\text{-}1}$ 206.4) at 55°, but only an upper limit of its percentage in solution (≤0.03 mol-%) is possible due to low spectral s/n ratio. Because of the uncertainty in the proportion of aldehyde, equilibrium constants for each component reaction of apiofuranose anomerization are subject to considerable error. Consequently, ring-closing rate constants derived from equilibrium constants and ring-opening rate constants are not reliable and are not considered in this discussion. However, because the ring-opening rate constants for **1**–**4** are *similar in magnitude* to those for **7** and **8** (see Table VI), and as the percentage of carbonyl form in solution is considerably smaller for **11** than for **7** and **8**, we conclude that

ring-closing rate constants must be considerably greater for **1–4** than for **7** and **8**; this is consistent with the known effect of substitution on ring-closure rates[33b,c].

Because of the structural relationships between apiofuranoses **1–4** and the tetrofuranoses **7** and **8**, a comparison of ring-opening rate constants for these compounds may reveal important factors that affect this reaction. Apiofuranose ring-opening rate-constants were determined by saturation-transfer ^{13}C-n.m.r. spectroscopy[6,8,21a] (see Table VI); the results of previous measurements[6] on **7** and **8** under the same solution conditions are included in Table VI for comparison. These data illustrate the effect of replacement of H by CH_2OH at C-3 of the tetrofuranose ring. Ring-opening rate constants for the 3-*C*-(hydroxymethyl)-L-threofuranose anomers are significantly different, with the *cis*-O-1,O-2 anomer (**3**) opening faster than the *trans*-O-1,O-2 anomer (**4**); the same is true for **8**. In contrast to erythrose (**7**), however, D-apiofuranoses **1** and **2** open at more similar rates, approximately equal to that observed for 3-*C*-(hydroxymethyl)-α-L-threofuranose (**4**).

The relative rates of ring-opening of uncharged furanose anomers are sensitive to the number and relative configuration of substituents in the molecule. The similar ring-opening rate constants for **1** and **2** (see Table VI) suggest that a 1,2-interaction between OH groups, and a 1,3-interaction between OH and CH_2OH, have a similar effect on ring-opening. The 1,2-interaction between OH and CH_2OH in **4** appears to be more kinetically destabilizing than any single substituent interaction in apiofuranose rings; by comparison, the 1,3-OH–OH interaction is probably a relatively minor factor in destabilizing **4**. From these considerations, it is expected that two 1,2-interactions (OH–OH, OH–CH_2OH) and a 1,3-interaction (OH–CH_2OH) might make **3** more *kinetically* unstable than **1**, **2**, and **4**, causing an enhanced rate of ring-opening. This expectation is realized (see Table VI). It is not clear at present why certain interactions result in kinetically destabilized rings with respect to ring-opening. This kinetic instability does not always reflect thermodynamic instability; as discussed previously[8], there is no valid reason to expect such a correlation. More appropriately, it is important to consider the difference in energy between the most favored conformation of the putative protonated intermediate and that of the transition-state structure for ring-opening; presumably, this energy difference is smaller for **3** than for **1**, **2**, and **4**.

From studies to date, the preference for ring-opening by α *anomers* of the pentofuranose 5-monophosphates[8] appears to be a unique characteristic of these compounds. The intramolecular mechanism of phosphate-catalyzed anomerization at pH ~5.0 seems relatively insensitive to furanose-ring configuration other than at C-1 and C-4. This α-furanose rule[44] does not appear to hold for uncharged furanoses. In the absence of intramolecular catalysis, the mechanism of furanose anomerization can discriminate between the apparently more subtle stereochemical and conformational features of the linear and cyclic forms.

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