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SYNTHESIS OF L-(4-^{2}H)ERYTHROSE, L-(1-^{13}C, 5-^{2}H)ARABINOSE AND L-(2-^{13}C, 5-^{2}H)ARABINOSE AND IDENTIFICATION OF THE INTERMEDIATES BY ^{2}H AND ^{13}C-N.M.R. SPECTROSCOPY*

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ABSTRACT

L-(1-^{13}C, 5-^{2}H)Arabinose (**6D**) and L-(2-^{13}C, 5-^{2}H)arabinose (**8D**) have been synthesized by degradation of 2,3-*O*-isopropylidene-α-L-rhamnofuranose (**2**) to L-(4-^{2}H)erythrose (**5β**, **5α**D), with subsequent chain elongation to **6D** plus L-(1-^{13}C, 5-^{2}H)ribose (**7D**), the latter being converted into **8D**. Intermediates were identified by complete assignment of the ^{13}C chemical shifts employing carbon–carbon and carbon–deuterium coupling constants, deuteration shifts, differential isotope-shifts, and deuterium spectra. The anomeric carbon atoms of **2** and 2,3-*O*-isopropylidene-L-(1-^{2}H) erythrose (**4D**) gave only single ^{13}C resonances, suggesting that these two compounds exists in only one major anomeric configuration, clarifying previously reported work. The synthesis of 2,3-*O*-isopropylidene-L-(1-^{2}H)rhamnitol (**3D**) facilitated the assignment of the signals in the ^{13}C spectra of the nondeuterated analog. Specific deuterium-enrichment and the observed carbon–deuterium coupling ($^1J_{C,D}$ ~22 Hz) not only served to identify the deuterated carbon atom unambiguously in **3** but also permitted assignment of closely spaced resonances. The deuterium spectrum of 2,3-*O*-isopropylidene-L-(1-^{2}H)erythrofuranose (**4D**) showed only a single resonance, indicating preponderance of one anomer, in accord with the observation of a single C-1 resonance in the ^{13}C spectrum.

INTRODUCTION

Plant cell-wall synthesis is an integral part of the metabolism associated with growth. D-Glucose, L-arabinose, and D-xylose are major sugars that are incorporated into the cell-wall polysaccharides of monocotyledonous plants. Accumulation of radioactivity from arabinose into the cell-wall polysaccharides of *Zea mays* coleoptiles has been demonstrated[1]. Recently Sillerud *et al.*[2] have shown that L-arabinan and β-D-glucan are synthesized from (1-^{13}C)glucose and that the ^{13}C incorporation into β-D-glucan may be monitored *in vivo* by ^{13}C-n.m.r. in millet sus-

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pensions. ^{13}C-N.m.r. shows considerable promise for the study of the regulation of polysaccharide synthesis *in vivo*, but suitable specifically ^{13}C-enriched precursors are required. *In vivo* synthesis of L-arabinan in plants from ^{13}C-labeled L-arabinose may also be monitored by ^{13}C-n.m.r. We therefore undertook the synthesis of L-(1-^{13}C)- and L-(2-^{13}C)arabinose to study their *in vivo* incorporation into cell-wall arabinan.

L-(1-^{13}C)Arabinose was prepared from L-erythrose[3]by condensation with (^{13}C)cyanide[4–6]; L-erythrose was obtained by degradation of L-rhamnose[3]. Although intermediates in the synthesis of L-erythrose have been previously identified by tedious physical and chemical methods, assignments for their anomeric configurations have remained ambiguous[7,8]. As erythrose is rare and is an important source for L-(1-^{13}C)arabinose, its derivation from L-rhamnose is of interest for an improved synthesis of L-erythrose.

The ^{13}C-n.m.r. spectrum of 2,3-*O*-isopropylidene-L-rhamnose (**2**) showed a single resonance from the anomeric carbon atom, and the ^{1}H-n.m.r. spectrum[9] of **2** likewise gave a single H-1 signal, suggesting that only one anomer of **2** is present. Although previous reports[3c,7,8] have suggested the existence of more than one anomer, our results suggest that only the β anomer is present. For unambiguous assignment of the chemical shifts of several carbon atoms, the deuterated analogs of various structures have been prepared.

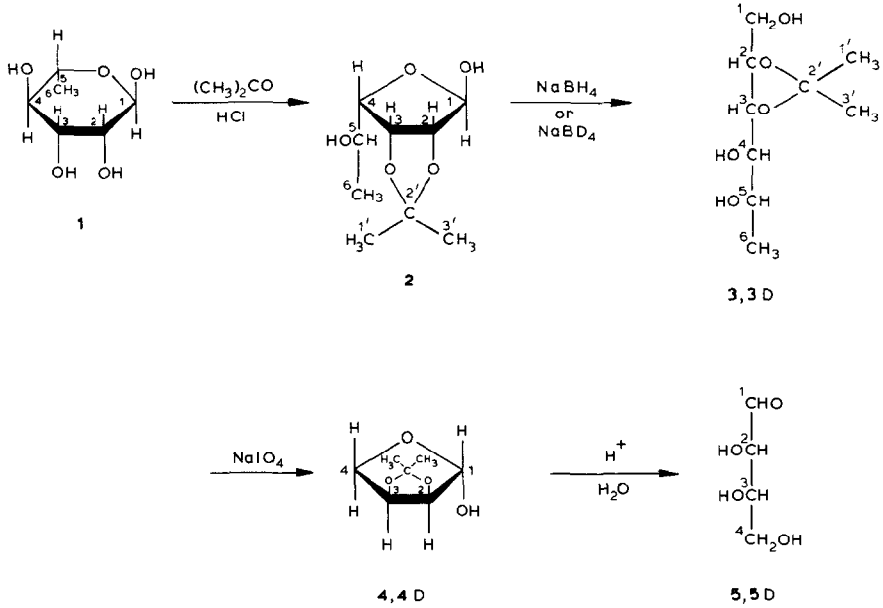
RESULTS AND DISCUSSION

Synthesis. — L-(4-^{2}H)Erythrose (**5**α,β**D**)* was prepared from α-L-rhamnose (**1**) by the method of Baxter and Perlin[3c] (Scheme I). Acetonation of **1** to yield 2,3-*O*-isopropylidene-β-L-rhamnose (**2**) followed by reduction with sodium borohydride gave 2,3-*O*-isopropylidene-L-rhamnitol (**3**). Reduction of **2** with sodium borodeuteride gave 2,3-*O*-isopropylidene-L-(1-^{2}H)rhamnitol (**3D**). Compounds **3** and **3D** were converted by sodium periodate into **4** and **4D**, respectively. Acid hydrolysis of these yielded L-erythrose (**5**β, **5**α) and L-(4-^{2}H)erythrose (**5**β**D**, **5**α**D**), respectively. The deuterated analogs were utilized for assigning ^{13}C resonance signals.

L-(1-^{13}C, 5-^{2}H)Arabinose (**6D**) was prepared from L-(4-^{2}H)erythrose (**5D**) (Scheme II). The Kiliani–Fischer synthesis gave L-(1-^{13}C, 5-^{2}H)arabinose (**6D**) and L-(1-^{13}C, 5-^{2}H)ribose (**7D**) through condensation of (^{13}C)cyanide with **5D**.

The syrupy product from the epimerization of **7D** was treated with Dowex 50W (Ba^{2+}) resin to give L-(2-^{13}C, 5-^{2}H)arabinose **8D** (83.7%) and traces of L-(2-^{13}C, 5-^{2}H)xylose (**9D**, 11.6%), L-(1-^{13}C, 5-^{2}H)lyxose (**10D**, 3.1%), and L-(1-^{13}C, 5-^{2}H)arabinose (**6D**, 1.6%) (Scheme II; see also Fig. 7C). The relative amount of each product was estimated based on the integrals of the ^{13}C-labeled carbon atoms.

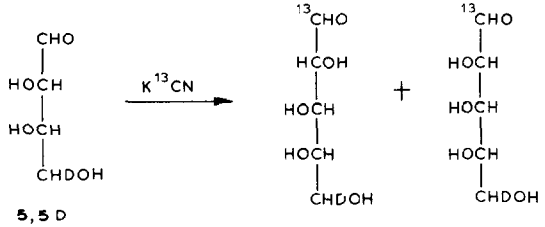
*Compounds having deuterium incorporation are here distinguished by the D designator following the compound number.



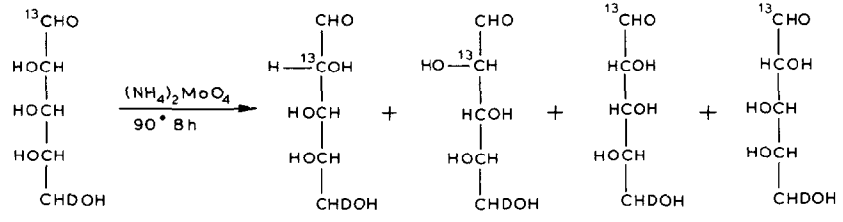
1 2 3,3 D 4,4 D 5,5 D

Scheme I

5β, 5βD 5α, 5αD



5,5 D 6,6 D 7,7 D



7,7 D 8,8 D 9,9 D 10,10 D 6,6 D

Scheme II

The formation of the C-3 epimer **9D** and the 2,3-diepimer **10D** upon epimerization of **7D** for a long period is in agreement with Hayes *et al.*[10], although the mechanism of the formation of L-(1-^{13}C, 5-^{2}H)arabinose (**6D**) is obscure. The material crystallized from syrupy product **9D** gave a ^{13}C spectrum of L-(2-^{13}C, 5-^{2}H)xylose (**9D**) containing traces of **6D** (1.7%) and **10D** (0.6%) on the basis of the integrals of the respective peaks (see Fig. 7A, B).

Carbon-13 n.m.r. assignments. — *2,3-O-Isopropylidene-β-L-rhamnose* (**2**). A natural-abundance ^{13}C-n.m.r. spectrum showed 9 distinct signals (Fig. 1 and Table I). The resonance at 100.88 p.p.m. was assigned to C-1 of the furanose form, based on the fact that C-1 of furanoses resonates 4–5 p.p.m. downfield relative to the C-1 shifts of the corresponding pyranoses[11]. The fact that only one signal for C-1 is present indicated the presence of only one anomer. Serianni *et al.*[5] observed and we confirmed (in the L series) that C-1 of β-D-erythrose resonates at 102.40 p.p.m. and that of α-D-erythrose at 96.80 p.p.m. The C-1 resonance of **2** at 100.88 p.p.m. was thus assigned to the β anomer.

The signals at 24.75 and 26.05 p.p.m. were tentatively assigned to the nonequivalent CH_3 carbon atoms above and below the plane of the 1,3-dioxacyclopentane ring, respectively.

The C-2′ signals of **2, 3,** and **3D** were assigned on the basis of their lack of directly bound protons. The resulting lack of n.O.e. and attendant long T_1 is characteristic. The T_1 values for compound **3** (see Table II) show that the signal at 110.19 p.p.m. has a T_1 value (11.9 sec) that is at least 10 times greater than that for any other carbon resonance in the molecule. This signal is thus assigned to that of C-2′ of **3**, **3D**, and similarly the signal at 112.73 p.p.m. is that from C-2′ of **2**.

Based on the C-4 and C-5 signals of methyl β-D-ribofuranose, which are known to resonate at 83.0 and 62.9 p.p.m., respectively[12], and from the d.i.s. values of **2** (Table II) described later, the resonance at 66.42 p.p.m. was assigned to C-5.

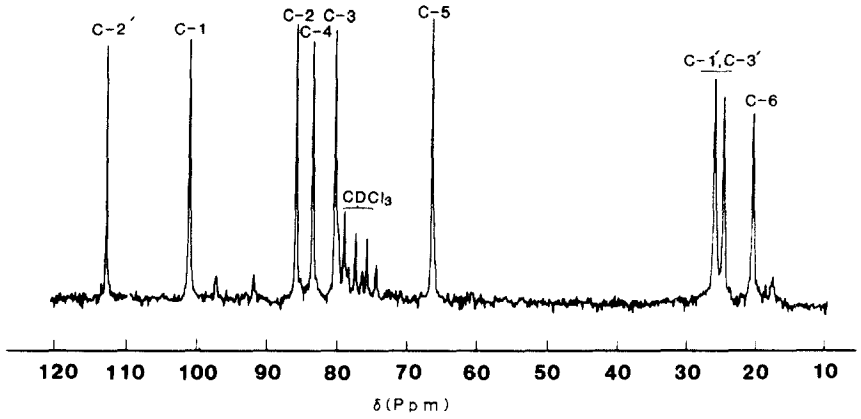


Fig. 1. The proton-decoupled ^{13}C-n.m.r. spectrum of 2,3-*O*-isopropylidene-β-L-rhamnose (**2**). No signals were observed downfield of C-2′. Minor resonances were caused by β-L-rhamnose.

TABLE I

ASSIGNMENT OF SIGNALS IN ^{13}C-N M R SPECTRA OF INTERMEDIATES, IN δ (p.p.m.)[a] AND ^{13}C–^{2}H COUPLING CONSTANTS ($^{1}J_{C,D}$) IN Hz

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-2'*	*C-1', C-3'*
1[b] α	95.0	71.9	71.1	73.3	69.4	18.0	—	—
β	94.6	72.4	73.8	72.9	73 1	18.0	—	—
2	100.88	85.75	80.23	83.37	66.42	20.52	112.73	24.75, 26.75
3, 3D[c] ($^{1}J_{C,D}$= 21.5)	62.20	78.72	76.87	73.76	68.84	20.07	110.19	25.85, 27.85
4,4D	101.65	85 22	79.91	71.61 (71.30)[d] ($^{1}J_{C,D}$ = 22.3)			112.14	24.65, 26.05
5, 5D α	96.81	72.43	70.63	72.66 ($^{1}J_{C,D}$ = 22.4)				
β	102.40	77.71	71.74	72.43 ($^{1}J_{C,D}$ = 22.4)				
h	90.75	74.88	73 24	63.96 (63.42)[d] ($^{1}J_{C,D}$ = 22.2)				
5-1[e] α	96.80	72.40	70.60	72.90				
β	102.40	77.70	71.70	72.40				
h	90.80	74.90	73.00	64.00				

[a]Chemical shifts are references to external Me_4Si. [b]Ref. 15. [c]The corresponding deuterated compound. [d]Chemical shifts of deuterated carbon atom. [e]Ref. 5.

A d.i.s. value of 0.04 p.p.m. (γ effect)[13] was observed for the signal at 83.37 p.p.m. (see Table II) and this signal was assigned to C-4.

The large steric perturbation that occurs at C-4 when **2** is converted into **3D** (Scheme I) is expected to be accompanied by a large shift of the C-4 resonance. Thus, C-4 of **2** should resonate at 83.37 p.p.m., whereas C-5, which is the least perturbed, should resonate at 66.42 p.p.m.

Table II shows that the pair of resonances at 85.75 and 80.23 p.p.m. is similar to a pair of resonances at 85.22 and 79.91 p.p.m. of the furanose moiety of **4**. Gorin and Mazurek[7] observed that the resonance of the carbon atom adjacent to the anomeric carbon of methyl pentofuranosides is shifted 2–5 p.p.m. downfield. From these data and a 0.05 p.p.m. d.i.s. at 85.75 p.p.m., the signal was assigned to C-2, whereas C-3 should resonate at 80.23 p.p.m.

*2,3-O-Isopropylidene-*L*-rhamnitol* (**3**) *and 2,3-O-isopropylidene-*L*-(1-*^{2}*H)rhamnitol* (**3D**). Assignments for **3D** are based on the deuterium-isotope effect and on a comparison of the resonances of **2** with those of **4D**. Reduction of **2** by sodium borodeuteride causes a large perturbation of the C-1 and C-4 chemical environment, and a large shift in the resonances for C-1 and C-4 was expected. A resonance at 62.20 p.p.m. was unequivocally assigned to C-1 based on the observation of a triplet with a coupling constant of 21.5 Hz ($^1J_{C,D}$) (Table I) at this field in the ^{13}C spectrum of **3D** (Fig. 2).

Deuterium-induced isotope shifts (d.i.s.)[13] were measured as an aid in the assignment of the chemical shifts of the hydroxylated carbon atoms 1, 4, and 5 of **3**. Carbon-13 spectra were measured simultaneously in the magnetic field with a dual coaxial n.m.r. cell (D_2O *vs.* H_2O environment) as described by Pfeffer *et al.*[13]. The d.i.s. values for the carbon resonances at 62.20, 73.26, and 69.84 p.p.m. were −0.09, −0.09 and −0.12 p.p.m., respectively (Table II); d.i.s. values for all the other ^{13}C resonances were zero. This suggests that these 3 signals arise from

TABLE II

DIFFERENTIAL ISOTOPE SHIFTS (D.I.S.), DEUTERATION SHIFTS (Δ), AND SPIN-LATTICE RELAXATION TIMES (T_1) FOR COMPOUNDS **2**, **3**, AND **4**

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-2′*	*C-1′, C-3′*
2 δ[a]	100.88	85.75	80.23	83.37	66.42	20.52	112.73	24.75, 26.05
D.i.s. (p.p.m.)	−0.11[b]	0.05	0	0.04	−0.13		0	0, 0
3 δ	62.20	78.72	76.87	73.26	69.84	20.07	110.19	25.85, 27.85
D.i.s. (p.p.m.)	−0.09	0	0	−0.09	−0.12		0	0, 0
Δ	−0.34[b]	−0.06	0	0	0	0	0	0, 0
T_1(sec)[c]	0.59	0.78	0.88	0.76	0.69	0.88	11.91	1.07, 0.91
4 δ	101.65	85.22	79.91	71.61			112.14	24.65, 26.05
Δ (p.p.m.)	0	0	−0.06	−0.31			0	0, 0

[a]Chemical shifts are in p.p.m. relative to external Me_4Si. [b]Minus sign indicates upfield shift. [c]Spin–lattice relaxation times were measured by means of an inversion-recovery sequence (180° − t − 90°)

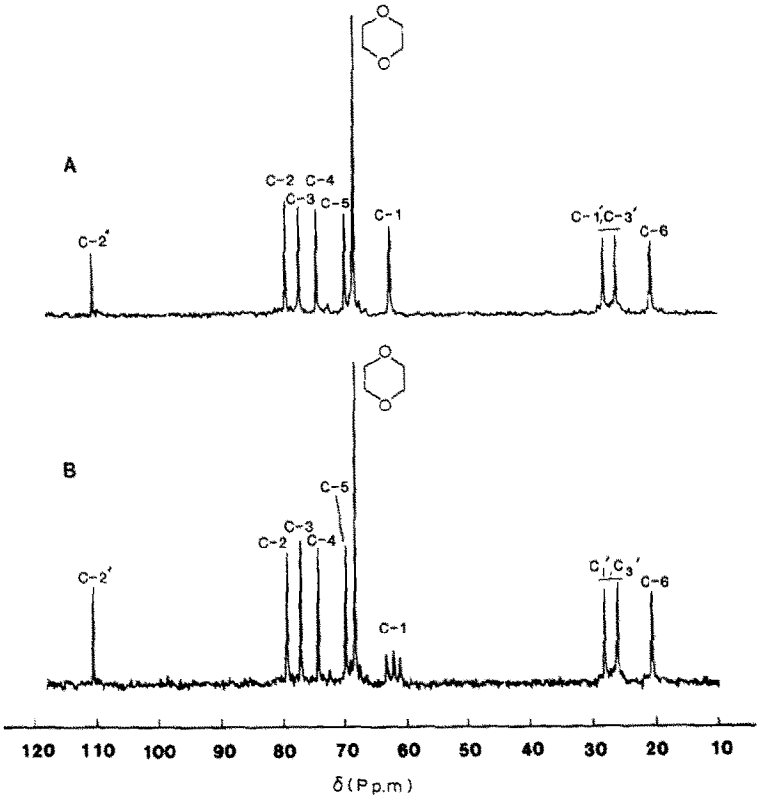


Fig. 2. The proton-decoupled ^{13}C-n.m.r. spectrum of (A) 2,3-*O*-isopropylidene-L-rhamnitol (**3**) and (B) 2,3-*O*-isopropylidene-L-(1-^{2}H)rhamnitol (**3D**).

hydroxyl-bearing carbon atoms and that the resonance at 73.26 p.p.m. should be from C-4 of **3**. A large upfield shift (10 p.p.m.) was observed for C-4 of **2** and from C-4 of **2** upon opening the ring to form **3**.

Based on the fact that there was only a small change in the chemical environments for C-5 and C-6 of **2** when the ring was opened to form **3**, and the expected small effect on the resonances (Table I), the signal at 69.84 p.p.m. was assigned to C-5 and that at 20.07 p.p.m. to C-6.

Observation of an upfield deuteration-shift of only 0.06 p.p.m. (Table II), of the signal at 78.72 p.p.m. of **3** in the ^{13}C-n.m.r. spectrum of a mixture of deuterated (**3D**) and non-deuterated (**3**), led to assignment of this signal to C-2 of **3** rather than[14,15] C-3. A similar β isotope-shift of 0.07 p.p.m. for C-3 was observed from methyl 2-*O*-methyl-β-D-(2-^{2}H)ribofuranoside[14]. The remaining resonance at 76.86 p.p.m. was assigned to C-3 of **3**.

*2,3-*O*-Isopropylidene-*L*-erythrose* (**4**) *and 2,3-*O*-isopropylidene-*L*-(4-^{2}H)erythrose* (**4D**). The ^{13}C-n.m.r. spectrum of the periodate-cleavage product, 2,3-*O*-isopropylidene-L-erythrose (**4**), consisted of 7 signals (Fig. 3A). Although a crystalline

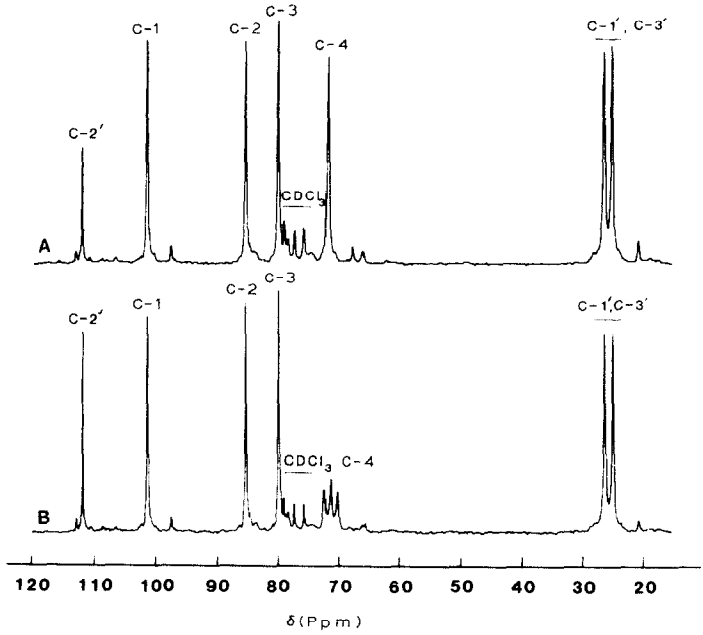


Fig. 3 The proton-decoupled ^{13}C-n.m.r. spectrum of (A) 2,3-*O*-isopropylidene-β-erythrofuranose (**4**) and (B) 2,3-*O*-isopropylidene-β-(4-^{2}H)erythrofuranose (**4D**).

material having m.p. 29–31° has been reported[3c], we have assigned the ^{13}C chemical shift of the signals from the syrup for the convenience of continuing directly with the hydrolysis step.

A triplet at 71.61 p.p.m. with an observed coupling-constant of $^{1}J_{C,D}$ = 22.3 Hz (Table I) of the deuterated analog (**4D**) was unequivocally assigned to C-4 of **4D** (Fig. 3B). The resonances from **4** were compared with the signals from **2**. The signals at 101.65, 85.22, and 79.91 p.p.m. from **4** are close to the resonances from C-1 (100.88 p.p.m.), C-2 (85.75) and C-3 (80.23) of **2**, respectively. Based on this comparison, we assigned the signal from C-1 to 101.65, C-2 to 85.22, and C-3 to 79.91 p.p.m. (Table I).

The β-isotope shift of 0.06 p.p.m. (Table II) for the signal at 79.91 p.p.m. further indicates that this resonance was from C-3 of **4D** and the resonance at 71.61 p.p.m. was from the deuterated carbon (C-4). These two resonances are thus unambiguously assigned. Gorin and Mazurek[14] reported that the carbon atom adjacent to the anomeric carbon of a furanose ring resonates 3–5 p.p.m. downfield of the resonance from C-2. On this basis, the assignment of the resonance at 85.22 p.p.m. to C-2 was reaffirmed. Serianni *et al.*[5] reported a 5.6-p.p.m. downfield shift for the resonance from C-1 of β-D-erythrofuranose as compared with the anomeric carbon resonance of α-D-erythrofuranose. The single peak at 101.65 p.p.m. was thus assigned to C-1 of the β-L anomer of **4D**.

L-*Erythrose and* L-*(4-*^{2}*H)erythrose* (**5α,β** *and* **5α,βD**). Acid hydrolysis of 2,3-

O-isopropylidene-L-erythrose and purification of the hydrolyzate by column chromatography on Dowex 50W (Ba^{2+}) gave a ^{13}C-n.m.r. spectrum free from the signals of the *O*-isopropylidene (Fig. 4) and identical with the natural-abundance ^{13}C-n.m.r. spectrum of D-erythrose[5]. The spectrum contained 10 peaks, indicating the existence of more than one tautomer, unlike that from 2,3-*O*-isopropylidene-L-(1-^2H)erythrose (**4D**), and suggesting that deacetonation allows both furanose anomers to exist to a measurable extent. Serianni *et al.*[5] assigned C-1β in β-L-erythrofuranose at 102.40 p.p.m., close to that observed here for C-1 of **4D**, confirming the β-configuration at C-1 of **4D**. The small upfield shifts of 7.51 and 8.17 p.p.m. for C-3 and C-2, respectively, that accompany deacetonation of **4** are in line with the foregoing suggestion (Table I).

A triplet centered at 63.42 p.p.m. in the ^{13}C spectrum of L-(4-^2H)erythrose (**5**α,βD, Fig. 4A). with observed coupling constants of $^1J_{C,D}$ = 22.2 Hz (Table I) indicated the presence of a deuterated carbon atom; the triplet was assigned to C-4 of the aldehydrol. The signals of C-4α and C-4β in **5**D were collapsed to overlapping triplets with an observed coupling constant of $^1J_{C,D}$ = 22.4 Hz (Table I) because of the deuterium at C-4. The resonances from C-2α,β and C-3α,β were readily assigned by comparison with reported chemical shifts[5] (Table I). The minor

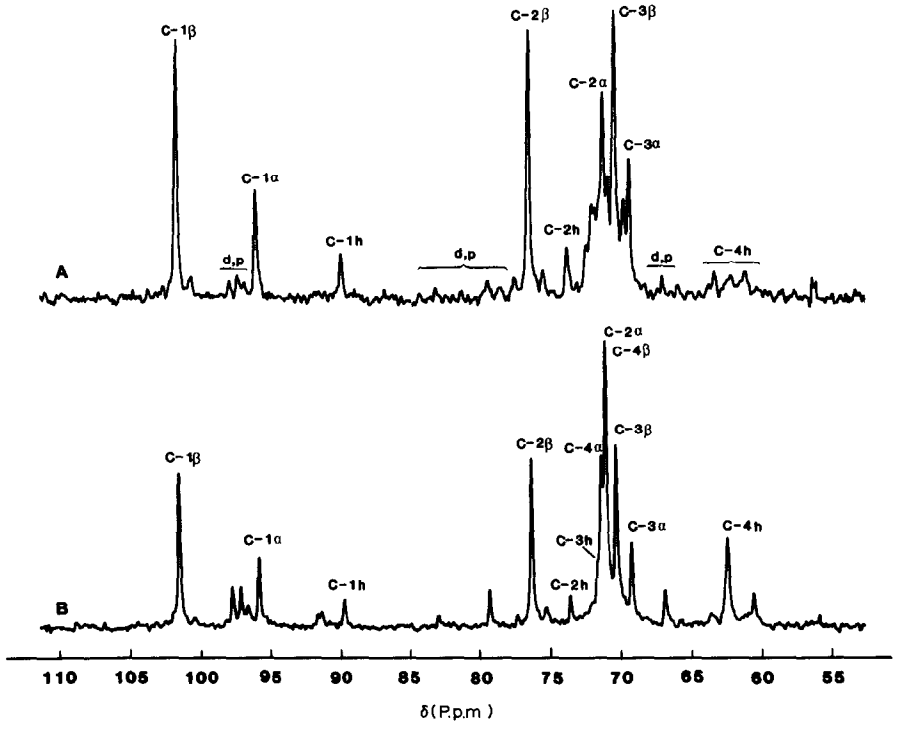


Fig. 4. The proton-decoupled ^{13}C-n.m.r. spectrum of (A) α,β-L-(4-^2H)erythrose (**5**D) and (B) α,β-L-erythrose (**5**). The symbol (d, P) denotes the dimer and polymer of erythrose and (h) denotes the aldehydrol form of L-erythrose.

TABLE III

ASSIGNMENT OF SIGNALS IN ^{13}C-N.M.R. SPECTRA OF L-(1-^{13}C, 5-^{2}H)ARABINOSE (**6D**), L-(1-^{13}C, 5-^{2}H)RIBOSE (**7D**), L-(2-^{13}C, 5-^{2}H)ARABINOSE (**8D**), ^{13}C–^{13}C COUPLING CONSTANTS ($^{1}J_{C,C}$) AND ^{13}C–^{2}H COUPLING CONSTANTS ($^{1}J_{C,D}$)

Compound		*C-1*		*C-2*		*C-3*	*C-4*	*C-5*	
		δ (p.p.m.)	$^{1}J_{C,C}$ *(Hz)*	*δ (p.p.m.)*	$^{1}J_{C,C}$ *(Hz)*	*δ (p.p.m.)*	*δ (p.p.m.)*		$^{1}J_{C,D}$ *(Hz)*
6D	α	97.80[a]	45.6	73.05		63.59[b]	69.72[b]	67.33 (67.04)[c]	21.6
	β	93.63	45.4	69.68		69.53[b]	69.53[b]	63.48 (63.19)[c]	21.6
7D	α	94.45	45.0	71.03		70.24[b]	68.26[b]	63.89 (63.59)[c]	22.4
	β	94.80[a]	45.0	72.05		69.92[b]	68.26[b]	63.89 (63.59)[c]	22.4
	fα	97	[d]						
	fβ	102	[d]						
	fβ	102	[d]						
8D	α				45.7			(67.04)[c]	21.6
					45.8			(63.19)[c]	21.9

[a]Chemical shifts of **6D** were referenced to C-1α of L-arabinose set to 97.80 p.p.m. downfield from Me_4Si, and of **7D** were referenced to C-1β of L-ribose set to 94.80 p.p.m. [b]Ref. 12. [c]Chemical shifts of deuterated carbon. [d]Carbon–carbon couplings were observed but could not be reliably measured

peaks at 98.69, 98.12, 92.73, 92.38, 84.11, 80.38, and 68.16 p.p.m. (Fig. 4, A and B) of the spectrum were enhanced in intensity in concentrated solutions of L-erythrose. These peaks were also observed during the preparation of D-erythrose[5]. Erythrose in solution readily enters into loose intermolecular association, possibly with formation of dimers or other complexes[3b]. The Kiliani–Fischer reaction for this mixture of erythrose tautomers, however, gave the same result as that for monomeric furanose[16].

L-(1-^{13}C, 5-^{2}H)Arabinose (**6D**) *and L-(1-^{13}C)arabinose* (**6**). A pair of triplets centered at 67.04 and 63.19 p.p.m. with observed coupling constants of $^{1}J_{C,D}$ = 21.6 Hz (Table III) in the spectrum (see Fig. 6A, B) of L-(1-^{13}C, 5-^{2}H)arabinose (**6D**) were unambiguously assigned to C-5α and C-5β respectively, of **6D**. The coupling constants of 45.4 and 45.6 Hz (Table III) for C-1β to C-2β and C-1α to C-2α are in agreement with reported data (~46 Hz)[17]. The assignment for C-2β was based on the coupling between C-1β and C-2β. Different assignments were reported for C-2, C-3, and C-4 of β-arabinose by earlier workers[15,18], but these signals are resolved by at most only 0.2 p.p.m. The signal at 69.68 p.p.m., however, was unambiguously assigned to C-2β of β-L-(1-^{13}C)arabinose (Table III) based on the observed spin-coupling of C-1β to C-2β. The signal at 69.53 p.p.m. was the unresolved sum of the C-3β and C-4β resonances, based on reported data[14,15,18–20]. The peak at 69.72 p.p.m. was then assigned to C-4α. Observation of the spin coupling of C-1α indicated an enrichment value of ~96% ^{13}C at C-1 (see Fig. 6B).

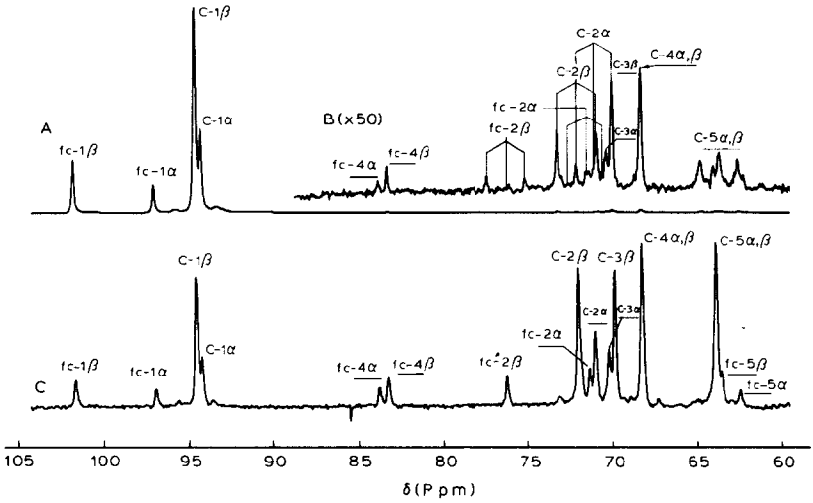


Fig. 5. The proton-decoupled ^{13}C-n.m.r. spectra of (A, B) L-(1-^{13}C, 5-^{2}H)ribose (7D), and (C) the natural-abundance ^{13}C-n.m.r. spectrum of L-ribose. The symbol (f) denotes L-ribofuranose.

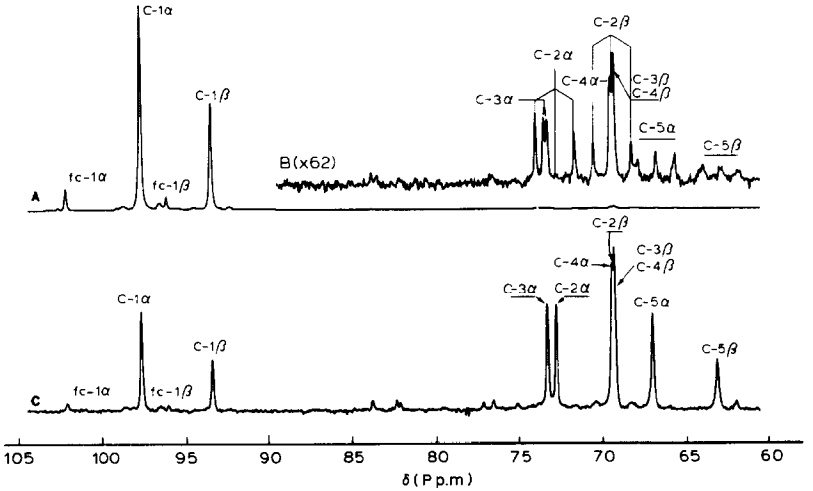


Fig. 6. The proton-decoupled ^{13}C-n.m.r. spectrum of (A, B) L-(1-^{13}C, 5-^{2}H)arabinose and (C) the natural-abundance ^{13}C-n.m.r. spectrum of L-arabinose. The symbol (f) denotes L-arabinofuranose.

L-(1-^{13}C, 5-^{2}H)Ribose (7D) *and L-(1-^{13}C)ribose* (7). The triplet at 63.59 p.p.m. in the ^{13}C-n.m.r. spectrum of 7D (Fig. 5) with the observed coupling constant of 22.4 Hz (Table III) at a single field indicates coupling of C-5α(β) to ^{2}H-5α(β), providing a basis for the unambiguous assignment of C-5α and β of L-(1-^{13}C)ribose. The signals at 72.05 and 71.03 p.p.m. were assigned to C-2β and C-2α respectively on the basis of the observed coupling-constants of C-1β to C-2β ($^{1}J_{C,C}$ = 45.0 Hz) and C-1α to C-2α ($^{1}J_{C,C}$ = 43.0 Hz) (Table III). Other resonances at 68.26, 69.92, and 70.24 p.p.m. were assigned to C-4α, C-4β, and C-3α by compari-

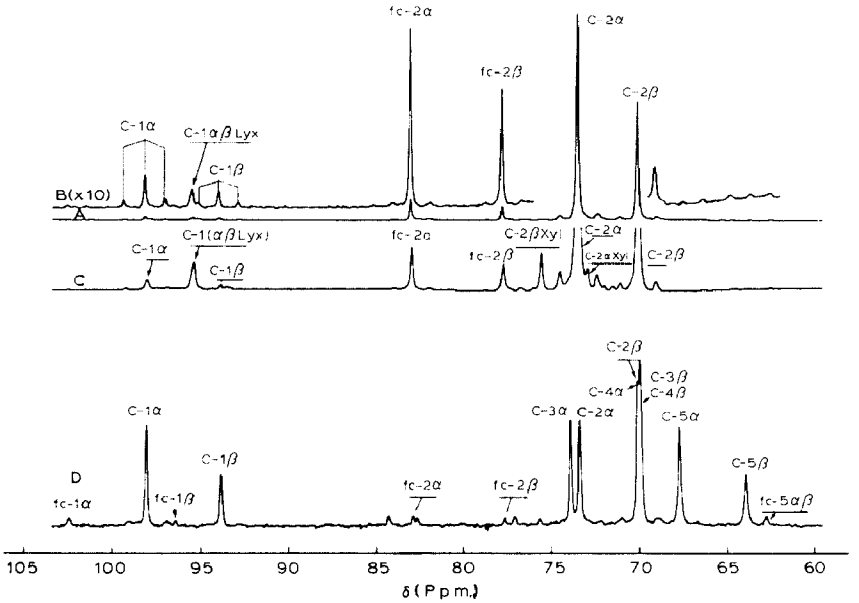


Fig. 7. The proton-decoupled ^{13}C-n.m r. spectrum of (A, B) L-(1-^{13}C, 5-^{2}H)arabinose (**8D**) contaminated by L-(1-^{13}C, 5-^{2}H)arabinose (**6D**, 1.7%) and L-(1-^{13}C)lyxose-5-^{2}H (**10D**, 0.6%). (C) The syrup from the preparation of **8D** contaminated by L-(1-^{13}C, 5-^{2}H)arabinose (**6D**, 1.6%), L-(2-^{13}C, 5-^{2}H)xylose (**9D**, 11.6%), and L-(1-^{13}C, 5-^{2}H)lyxose (**10D**, 3.1%). (D) The natural-abundance ^{13}C-n.m.r. spectrum of L-arabinose.

son with reported data[18,19,21,22]. The ^{13}C enrichment at C-1 was 97%, based on the C–C coupling of fC-1β to fC-2β (Fig. 5).

L-(2-^{13}C, 5-^{2}H)Arabinose (**8D**) *and L-(2-^{13}C)arabinose* (**8**). Carbon-13 labeling at C-2 permitted unequivocal assignment of the signal at 69.68 p.p.m. to C-2β, whose resonance was very close to resonances from C-3β and C-4α, C-4β. The unequivocal assignments of 82.66 and 77.44 p.p.m. (Fig. 7A, B) to C-2α and C-2β of the furanose agree with earlier assignments[11]. The couplings between C-2α and C-1α (45.7 Hz) and C-2β and C-1β (45.8 Hz, Table III) are in agreement with the reported data (~46 Hz)[17]. Enrichment of 93% at C-2 by carbon-13 was estimated on the basis of the peak height of the doublets arising from coupling of C-2α to C-1α and of C-2β to C-1β (Fig. 7B).

The prepared sample of L-(2-^{13}C, 5-^{2}H)arabinose (**8D**), also contained L-(1-^{13}C, 5-^{2}H)arabinose (1.7%) and traces of both L-(1-^{13}C, 5-^{2}H)lyxose (**10D**) and L-(2-^{13}C, 5-^{2}H)xylose (**9D**), as observed from the ^{13}C spectrum of the sample of L-(2-^{13}C, 5-^{2}H)arabinose (**8D**, Fig. 7B, C). The resonances at 72.65 and 75.25 p.p.m. were assigned to C-2α and C-2β, respectively, of L-(2-^{13}C)xylose[14,15,18,19,23] and that at 95.16 p.p.m. was assigned to C-1α and C-1β of lyxose[11,18].

Deuterium n.m.r. spectra of 2,3-O-isopropylidene-L-(1-^{2}H)rhamnitol (**3D**), *2,3-O-isopropylidene-β-L-(4-^{2}H)erythrofuranose* (**4D**) L-*(4-^{2}H)erythrose* (**5α,βD**), L-*(1-^{13}C, 5-^{2}H)arabinose* (**6D**), L-*(1-^{13}C, 5-^{2}H)ribose* (**7D**), *and* L-*(2-^{13}C, 5-*

TABLE IV

CHEMICAL SHIFTS OF SIGNALS IN THE ^{2}H-N M R SPECTRA OF INTERMEDIATES

	3D	4D	5D	6D	7D	8D
δ (p.p.m.)[a]	3.48	4.10	3.57 3.99	3.48 3.69	3.49	3.44 3.59

[a]Chemical shifts are reported relative to a D_2O internal standard set to 4.50 p.p.m.

^{2}H)arabinose (**8D**). Chemical shifts of the deuterium n.m.r. spectra of the intermediates and the products are shown in Table IV. The deuterium spectra of **3D** and **4D** showed single resonances at 3.48 and 4.10 p.p.m. respectively, indicating that the deuterons at C-1 of **3D** and C-4 of **4D** exist in a single chemical environment. This is in accordance with our observation of a single resonance from C-1 in the ^{13}C spectrum of **3D** and from C-4 of **4D** (Figs. 2 and 3). The D-n.m.r. spectra of **5D**, **6D**, and **8D** showed partially resolved, unsymmetrical doublets (Table IV), suggesting that the deuterium signals arise from α and β anomers of these compounds. This is analogous to our observation of well-resolved ^{13}C signals from C-4α and C-4β of **5**α,βD and C-5α and C-5β of both **6D** and **8D** (Figs. 4, 6, and 7). The single resonance at 3.49 p.p.m. of the deuteron at C-5 of **7D** agrees with our finding of a single ^{13}C resonance from C-5$\alpha\beta$ (Fig. 5).

CONCLUSIONS

High-resolution ^{13}C-n.m.r. techniques have been used to characterize the intermediates in a synthesis of L-erythrose from α-L-rhamnose, and demonstrate that acetonation of α-L-rhamnose gives only the β-furanose anomer, thus resolving the question of the suspected α,β pair reported by earlier workers[3c,7,8]. The formation of only the β anomer was further substantiated by the ^{1}H-n.m.r. spectrum by Perlin[9] of 2,3-*O*-isopropylidene-L-rhamnose, which showed only a single anomeric-proton resonance. The absence of observed coupling between H-1 and H-2 suggests these protons to be orthogonal, as required for the β-furanose tautomer. Unequivocal anomeric assignment for **2** and **4** had not previously been made.

Two crystalline forms of mono-*O*-isopropylidene L-rhamnose (**2**) have been described having different melting points and specific optical rotations[7,8]. As these two forms mutarotate in aqueous solution to a constant value, $[\alpha]_D$ +17.8°, it was assumed that they constitute a pair of anomers. Levene and Compton[7] obtained two crystalline compounds only after the complete methylation of mono-*O*-isopropylidene-L-rhamnose, and they assumed that the two compounds were the α and β anomers.

Baxter and Perlin[3c], however, described only the β anomer of **2** during the synthesis of L-erythrose. The ^{13}C-n.m.r. spectrum of **2** in this report gives distinct signals (Fig. 1) which resolve this question. The single resonance observed near 100 p.p.m. (Fig. 1) indicates only one anomeric configuration of the furanose.

Synthesis of the deuterated analogs permitted unambiguous ^{13}C assignments for **3** and **4** (Scheme I), based on the effect of deuterium on the signals of the appended carbon atoms, which appear as triplets at higher field[12], and the effect on the signals of the adjacent carbon atoms, which are shifted upfield[11,12,14] by 0.06 p.p.m. (Table II). The hydroxylated carbon atoms were identified from the chemical-shift differences (0.09–0.12 p.p.m.) between the hydroxylated carbon atoms as observed in H_2O and D_2O (Table II). Complete interpretations of the spectra are presented in Tables I and III, and the values of the β-carbon shifts (deuterium substitution) and d.i.s. are summarized in Table II.

Examination of space-filling models shows that the favored bicyclic structure (Scheme I) of 2,3-*O*-isopropylidene-L-rhamnofuranose (**2**) has HO-1 *trans* to the substituent at C-4 of the furanose moiety and also *trans* to O-2, indicating that the β-furanose form is strongly favored[21]. Furthermore, H-bonding should occur between HO-5 and O-3, thus further stabilizing the molecule against reorientation[24] at C-1. This view may be significant in that Baxter and Perlin[3c] described only the β anomer of the 2,3-isopropylidene acetal of L-rhamnose (**2**, Scheme I). The putative α anomer of **2** isolated by earlier workers[7,8] could have been unreacted α-L-rhamnose, whose melting point (82–92°) is similar to that of the alleged α anomeric acetal (m.p. 79–80°).

The single resonance at 101.65 p.p.m. in the ^{13}C spectrum of **4** (Fig. 3) also indicates only one anomeric form, as already found for **2**. Inspection of models shows that HO-1 is least crowded when it is *trans* to O-2 (Scheme I). The assignment of the β-furanose structure[12] to **4** is further supported by comparison with data for the C-1 resonances of β-D-erythrose and of β-L-erythrose (**5**, **5**D) (Table I). No evidence for the presence of the α-furanose form was found for **4**, **4**D. In their synthesis of **4**, Baxter and Perlin[3c] did not indicate the anomeric form.

Carpita *et al.*[1] demonstrated that L-arabinose can be incorporated into polysaccharides of cell walls of *Zea mays* coleoptiles. On the basis of the ^{13}C-n.m.r. signal at 108.38 p.p.m. which arises from C-1 of L-arabinan, Sillerud *et al.*[2] have shown that arabinans are synthesized from D-(1-^{13}C)glucose in millet suspensions. Having prepared L-(^{13}C, 5-^{2}H)- and L-(2-^{13}C, 5-^{2}H)-arabinose, the incorporation of these ^{13}C sugars into cell-wall arabinan and other polysaccharides may be monitored *in vivo* by ^{13}C-n.m.r. thus allowing the study of the regulation of arabinan synthesis *in vivo*.

EXPERIMENTAL

Materials. — α-L-Rhamnose monohydrate was purchased from Sigma Chemical Co. (St. Louis, MO), and was dehydrated by heating *in vacuo* for 24 h at 50°. Sodium borodeuteride (88% ^{2}H) was purchased from MSD isotopes. Potassium (^{13}C)cyanide was supplied by Los Alamos National Laboratory, Group INC-4. Pd–$BaSO_4$ (5%) and ammonium molybdate were purchased from Sigma Chemical Co. All other chemicals were reagent grade and were used without further purification.

Instrumentation. — Proton-decoupled ^{13}C-n.m.r. spectra were obtained with a Varian CFT-20 n.m.r. spectrometer, operating at 20 MHz. Spectra were obtained with 8K data points in a spectral width of 4000 Hz (200 p.p.m.) with an acquisition time of 1.023 s. The spectrometer was locked to the resonance of D_2O contained in a coaxial capillary. Chemical shifts were measured relative to external tetramethylsilane. Spin–lattice relaxation times (T_1), deuterium-induced isotope shifts (d.i.s.)[13], and the chemical-shift changes upon deuteration[14] were determined with a Bruker WM-300 FT n.m.r. spectrometer, operating at 75.42 MHz using proton-noise decoupling. D.i.s. values were computed by subtracting the ^{13}C chemical shifts of the sample dissolved in H_2O from those found in D_2O. The deuteration shifts were computed by subtracting the ^{13}C chemical shifts of the protonated saccharide from those of the deuterated compound. Spectra were accumulated in 16K data points in a spectral width of 16,129 Hz (213.718 p.p.m.) with an acquisition time of 0.508 s.

2,3-O-Isopropylidene-β-L-rhamnofuranose (**2**). — A slight modification of the procedure of Perlin and Baxter[3c] and of Fischer[3a] was used. Finely powdered α-L-rhamnose (**1**, 60 g) was suspended in dry acetone (600 mL) in a 1-L round-bottom flask equipped with a stirring bar. A solution of acetone (40 mL) containing hydrogen chloride (3 g) was added and the mixture was stirred at room temperature until the solids dissolved (1 h). The mixture was cooled in ice. Ammonia was bubbled into the solution until no further solids were formed. The ammonium chloride was filtered off, and the filtrate evaporated. The syrupy residue was dissolved in water (100 mL), and extracted twice with ethyl acetate (200 mL). The extract was washed 4 times with water (100 mL), dried over sodium sulfate, and evaporated to give impure **2** as a clear syrup (44.35 g); $[\alpha]_D$ +4.6° (*c* 4.3, H_2O)[3c]; R_F 0.82 (t.l.c., cellulose, 40:11:19 BuOH–EtOH–H_2O) containing some starting α-L-rhamnose (R_F 0.43). Compound **2** was not obtained crystalline; the natural-abundance ^{13}C-n.m.r. spectrum of the syrup gave well defined signals for **2** and weak signals arising from α-L-rhamnose (Fig. 1).

2,3-O-Isopropylidene-L-(1-^{2}H)rhamnitol (**3D**). — Syrupy **2** (5.29 g) in water (30 mL) was added dropwise to a well stirred solution of sodium borodeuteride [2.04 g, in ice–water (45 mL)]. After 3 h at room temperature, acetic acid was slowly added to decompose the excess of sodium borodeuteride and adjust the pH to 6.0. The aqueous solution was evaporated to a clear syrup (4.3 g) whose ^{13}C-n.m.r. spectrum showed a triplet at 62.20 p.p.m. indicating deuterium incorporation (Fig. 2).

Treatment of the syrup with Dowex 50 (H^+) at pH 3 to remove the sodium acetate in the mixture and adjustment to pH 6.5 with Dowex 1-X2 (HCO_3^-) caused partial hydrolysis of the rhamnitol derivative.

2,3-O-Isopropylidene-β-L-(4-^{2}H)erythrofuranose (**4D**). — A solution of compound **2** (59.90 g) in 300 mL of water was added dropwise to a well stirred solution of sodium borodeuteride (18.2 g) in ice–water (450 mL). After 5 h at room temperature, acetic acid was slowly added to decompose the excess of borohydride and

adjust the pH to 6.3. The solution was cooled externaly to 10° and finely powdered sodium periodate (65.4 g) was added in portions during 2 h. The mixture was kept at room temperature, concentrated to 200 mL, and the slurry extracted four times with ethyl acetate (300 mL). The extract was washed twice with water (200 mL), dried over sodium sulfate, and evaporated to a clear syrup (37.8 g), whose ^{13}C-n.m.r. spectrum gave 6 peaks with a triplet at 71.30 p.p.m. indicative of deuterium incorporation at C-1 of **4D** (Fig. 3).

L-*(4-^{2}H)Erythrose* (*β*D *and* **5***α*D). — Compound **4D** (36.1 g) in 0.05M sulfuric acid (260 mL) was heated on a steam bath[3c]; $[\alpha]_D$ +0.842° (1 dm, initial)→0.582° (40 min, constant). After being made neutral with Dowex 1-X2 (HCO_3^-), the hydrolyzate was evaporated to a syrup (27.0 g). The ^{13}C-n.m.r. spectrum showed a triplet at 63.66 p.p.m. arising from the deuterated carbon atom at C-4 of the aldehydrol form (Scheme I, Fig. 4). Weak signals centered at 98, 84, and 67 p.p.m. were also observed[5]. The hydrolyzate was applied onto a column of Dowex 50W-X8

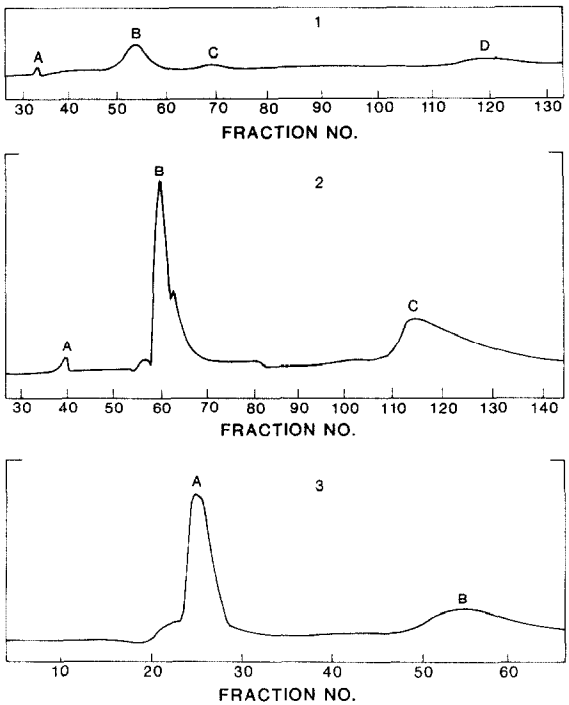


Fig. 8 (A) Chromatography of L-(1-^{2}H)erythrose (**5D**) on a column (9.5 × 155 cm) of Dowex 50W-X8 (200–400 mesh) in Ba^{2+} form using distilled water as the eluent. Peak A gave a salt and peaks B, C, and D gave α-L-rhamnose, L-rhamnitol, and L-erythrose, respectively. (B) Separation by ion-exchange chromatography as in (A) of L-(1-^{13}C, 5-^{2}H)arabinose (**6D**) and L-(1-^{13}C, 5-^{2}H)ribose (**7D**); 40-mL fractions. The peak A gave a salt, and peaks B and C gave **6D** and **7D**, respectively. (C) Separation of L-(2-^{13}C, 5-^{2}H))arabinose (**8D**) and L-(1-^{13}C, 5-^{2}H)ribose (**7D**) as in (A). Peak A gave L-(2-^{13}C, 5-^{2}H)arabinose (**8D**) containing small amounts of L-(1-^{13}C, 5-^{2}H)arabinose (**6D**), L-(1-^{13}C, 5-^{2}H)lyxose (**10D**), and L-(2-^{13}C, 5-^{2}H)xylose (**9D**). Peak B gave L-(1-^{13}C, 5-^{2}H)ribose (**7D**).

(Ba^{2+}). Fractions 111–132 gave 19.8 g of syrupy L-(4-^{2}H)erythrose (Fig. 8A). Fractions 48–60 and 67–73 gave L-rhamnose and L-rhamnitol, respectively.

L-(1-^{13}C, 5-^{2}H)Arabinose (**6D**) *and L-(1-^{13}C, 5-^{2}H)ribose* (**7D**). — L-(4-^{2}H)Erythrose (9.8 g) in water (20 mL) was added slowly to an aqueous solution (13 mL) of potassium (^{13}C)cyanide (5.98 g) according to Serianni *et al.*[4]. Fractions 50–72 from the Dowex 50W (Ba^{2+}) column used to resolve the Kiliani–Fischer reaction mixture (Fig. 8B) gave **6D** as a syrup (4.8 g) that crystallized almost completely when stored for 2 days at 4°; m.p. 155–159°; ^{13}C-n.m.r. spectrum see Fig. 6A, B. Fractions 105–140 afforded syrupy **7D** (4.5 g), which crystallized after 6 days at 4°; m.p. 80–84°; ^{13}C-n.m.r. spectrum see Fig. 5A, B.

L-(2-^{13}C, 5-^{2}H))Arabinose (**8D**). — L-(2-^{13}C, 5-^{2}H)Arabinose was prepared by epimerization of L-(1-^{13}C, 5-^{2}H)ribose by the method of Hayes *et al.*[10]. A solution of L-(1-^{13}C, 5-^{2}H)ribose (0.2M) and ammonium molybdate (10mM) was heated under reflux at 90°. The reaction was monitored every h by ^{13}C-n.m.r. After 8 h the ratio of the peak height of L-(1-^{13}C, 5-^{2}H)ribose to L-(2-^{13}C, 5-^{2}H)arabinose became constant. The mixture was cooled, treated with Dowex 50W (H^+) and then Dowex 1-X2 (HCO_3^-) to remove NH_4^+ and MoO_4^{2-}.

A mixture of the products (syrup, 6.8 g) was applied onto a column of Dowex 50W-X8 (Ba^{2+}). Fractions 47–70 (Fig. 8C) gave a syrup (2.2 g) whose ^{13}C spectrum was identical to that of L-(1-^{13}C, 5-^{2}H)ribose (Fig. 5, A and B). Fractions 15–35 were combined and evaporated to a syrup (3.2 g). The ^{13}C-n.m.r. spectrum (Fig. 7, C) of the syrup gave, in addition to the signals corresponding to that of L-(2-^{13}C)arabinose, 3 additional peaks at 72.65, 75.25, and 95.16 p.p.m. The syrup partially crystallized when kept overnight at 4°. The crystals were washed 3 times with cold methanol and dried under vacuum (2.1 g); ^{13}C-n.m.r. see Fig. 7, A and B.

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