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Preliminary communication

A ^{13}C-n.m.r. study on the conformation of L-ascorbic acid in deuterium oxide

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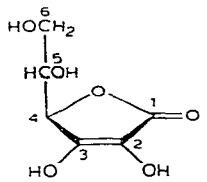
Aside from its significance as a vitamin and as an antioxidant in foodstuffs, recent biochemical studies on L-ascorbic acid (**1**) have disclosed its further importance in such biological roles as a co-factor of enzymes[1], and as a possible intermediate in biological sulfation[2] and in host resistance to malignant diseases[3].

As part of a project on chemical study of L-ascorbic acid, we now report ^{13}C-n.m.r. data for both L-ascorbic acid and 5-*C*-deuterated L-ascorbic acid[4] (**2**) that have revealed the preponderant conformation of **1** in aqueous solution.



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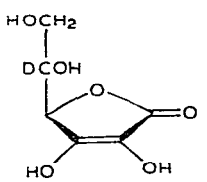

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The long-range, ^{13}C–^{1}H, spin–spin coupling-constants were obtained from the proton-coupled, ^{13}C-n.m.r. spectra with gated decoupling, and were confirmed either by long-range, selective, proton-decoupling experiments[5], or by comparison of the data for **1** and those for **2**, as shown in Figs. 1 and 2. The coupling constants thus obtained are summarized in Table I.

The observation of both a small, vicinal coupling $^{3}J_{C\text{-}4,H\text{-}6} = {}^{3}J_{C\text{-}4,H\text{-}6} = 2.4$ Hz, which indicates a *gauche* relationship[6(a)–(e)] between C-4 and H-6 and C-4 and H-6′ about the C-5–C-6 bond, and the normal, geminal coupling $^{2}J_{C\text{-}6,H\text{-}5} = 5.4$ Hz is consistent with rotamer **3** about the C-5–C-6 bond.

The observation of the small vicinal-couplings $^{3}J_{C\text{-}6,H\text{-}4} = 1.0$ Hz and $^{3}J_{C\text{-}3,H\text{-}5} = 1.5$ Hz, which indicate *gauche* relationships between both C-6 and H-4, and C-3 and H-5 about the C-5–C-4 bond, is consistent with either rotamer **4** or **5**. The small geminal-coupling of $^{2}J_{C\text{-}4,H\text{-}5} = 2.0$ Hz, compared to the normal value of 4–5 Hz, however, favors rotamer **4** (rather than **5**), as a *trans* relationship of H-5 to O-4 is present in **4** but a *gauche* one[6b] in **5**.

TABLE I

$^{13}C-^{1}H$ SPIN–SPIN COUPLING (Hz)[a]

Compound	*Coupling pathway*	*Dihedral angle (degrees)*	J_{vic}[b]	J_{gem}[b]	J_{direct}
1	^{13}C-1–O–C-4–H-4	0	2.0		
	^{13}C-2–C-3–C-4–H-4	60	2.0		
	^{13}C-3–C-4–C-5–H-5	60	1.5	5.4	
	^{13}C-4–C-5–C-6–H-6	60	2.0*	2.0*	152.8
	^{13}C-4–C-5–C-6–H-6′	60	2.0*	2.0*	152.8
	^{13}C-6–C-5–C-4–H-4	60	1.5	5.4	145.0
2	^{13}C-1–O–C-4–H-4	60	2.0		
	^{13}C-2–C-3–C-4–H-4	60	2.0		
	^{13}C-3–C-4–C-5–D			5.9	
	^{13}C-4–C-5–C-6–H-6	60	2.4*		
	^{13}C-4–C-5–C-6–H-6′	60	2.4*		
	^{13}C-6–C-5–C-4–H-4	60	1.0*		

[a] ^{13}C-N.m.r. data were obtained with a JNM-FX100 FT n.m.r. apparatus operated at 25.05 MHz at 25° for compounds dissolved in D_2O. δ_C are expressed in p.p.m. downward from tetramethylsilane. Conditions for FT measurements: spectral width, 5 KHz; repetition time, 3.6 sec.; number of data points, 16 K; power level of long-range, selective proton-decoupling ~ 800 nT. [b] All J_{vic} and J_{gem} values, except those marked with an asterisk, were confirmed by long-range, selective, proton-decoupling experiments, as shown in Figs. 1 and 2. Precision of J values is ±0.24 Hz.

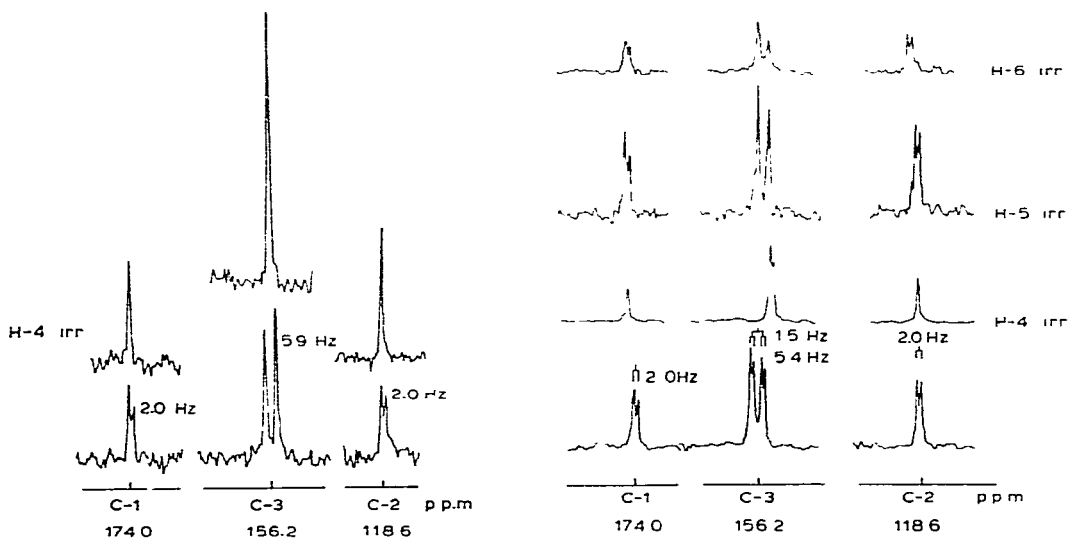


Fig. 1. Assignment of long-range, carbon–proton couplings through selective, proton decoupling (AA = ascorbic acid): left, proton-coupled spectra of 5-*C*-deuterated L-AA by gated decoupling technique; right, proton-coupled spectra of L-AA by gated decoupling technique.

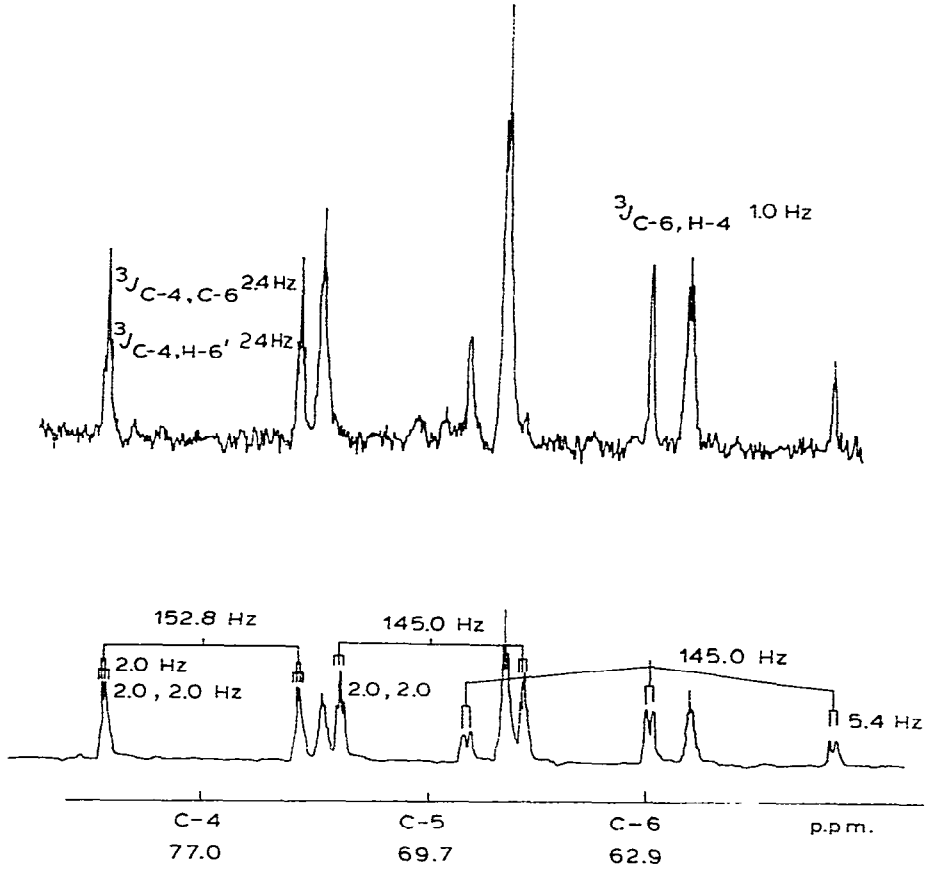
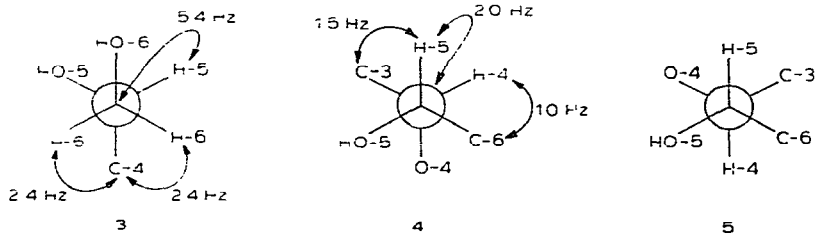
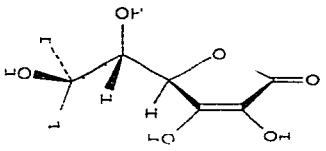


Fig. 2. Assignment of long-range, carbon–proton couplings through deuterium labelling (AA = ascorbic acid): top, proton-coupled spectra of 5-*C*-deuterated L-AA by gated decoupling technique; bottom, proton-coupled spectra of L-AA by gated decoupling technique.



Rotamers about the C-5—C-6 and C-5—C-4 bonds

In conclusion, the most favored rotamer of L-ascorbic acid in aqueous solution was deduced from the ^{13}C-n.m.r. data for **1** and **2**, and shown to be **6**.



6

Favored conformation of L-ascorbic acid

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