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Note

N.m.r. spectra of some 3,6-anhydro-D-galactose derivatives

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The widespread distribution of 3,6-anhydro-D-galactose or its enantiomorph in polysaccharides from varied species of the Rhodophyta has lately been confirmed[1–3]. In particular, the agars from certain origins have been found to be a family of polydisperse polysaccharides differing in their content of anhydrogalactose residues[4,5]. In addition to chemical methods, mass-spectrometric analysis of the anhydrogalactose derivatives has recently been reported to be useful for structural investigation of these polysaccharides[6]. The present work was undertaken to utilize the n.m.r. spectroscopic method also for this purpose. In the present note, the basic spectral data of some 3,6-anhydro-D-galactose derivatives are described, and discussed with respect to their conformational significance.

The n.m.r. spectrum of methyl 2,4-di-*O*-acetyl-3,6-anhydro-α-D-galactopyranoside (**1**) in chloroform-*d* shows complete separation of the signals for H-1, H-2, H-4, H-6, and H-6′, with overlap of the H-3 and H-5 signals; first-order analysis and assignment was readily made. The n.m.r. spectrum of methyl 3,6-anhydro-α-D-galactopyranoside (**2**) in deuterium oxide was more complicated, owing to partial overlapping of the signals of H-2 and H-6′, but it was successfully assigned by comparison with the spectrum of the acetylated derivative (**1**); it was assumed that both patterns would be similar except for the greater deshielding effects on H-2 and H-4 by the acetyl groups in **1**. N.m.r. parameters for compounds **1** and **2** are listed in Table I. The difference in τ-value of H-2 and H-4 between **1** and **2** is significant (0.9–1.1 p.p.m.), but there are no marked differences between **1** and **2** in the other parameters.

TABLE I

CHEMICAL-SHIFT AND FIRST-ORDER COUPLING DATA FOR DERIVATIVES OF 3,6-ANHYDRO-D-GALACTOSE

Compound	*Chemical shifts* (τ)							*Coupling constants* (*Hz*)						
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6′*	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
1	5.16	4.86	5.60	4.63	5.55	5.86	6.03	3.0	5.1	<0.5	1.9	<0.5	2.8	10.5
2	5.15	5.99	5.63	5.51	5.59	5.78	5.95	2.8	5.8	<0.5	2.0	<0.5	2.9	10.8
3	5.48	4.69	5.94	4.90	~4.87	6.06	5.98	7.3	3.7	3.8	~1.5	~2.2	~5.0	10.3
4	5.52	6.20	6.11	5.72–5.75		6.12	5.96	6.6	3.1	~4.7	2.8	<1.5	4.6	10.0

On comparing the n.m.r. parameters of **1** and **2** with those of the α-galactopyranose series[7], it was observed that introduction of the 3,6-anhydro ring caused a significant deshielding (~0.5 p.p.m.) of H-3, H-4, and H-6. The proton–proton coupling constants were also markedly influenced by formation of this ring, as revealed by the decrease in $J_{2,3}$ and $J_{3,4}$ (by 3–5 Hz) and the increase in $J_{4,5}$ (by 1–2 Hz). As the signals of all of the methylene and methine protons other than H-1 in D-galactopyranoses are known[7] to appear above τ 5.8, the lower-field signals of several protons (such as H-3, H-4, and H-5) in **2** may be utilized for analyzing the anhydro sugar in polysaccharides from red seaweeds.

The n.m.r. spectra of 2,4,5-tri-*O*-acetyl-3,6-anhydro-D-galactose dimethyl acetal (**3**) in chloroform-*d*, and 3,6-anhydro-D-galactose dimethyl acetal (**4**) in deuterium oxide, were also examined. Assignments of the signals in **3** were confirmed by spin-decoupling experiments. Initially, a doublet near τ 5.5 was assigned to H-1 from its broad spacing and low field-position. Irradiation of this signal collapsed a doublet of doublets near τ 4.7 into a doublet, which was thus identified as the H-2 signal. Irradiation of the H-2 signal, in turn, allowed the signal of H-3 to be located. Partially overlapping multiplets near τ 4.9 were regarded as the H-4 and H-5 signals because of their low τ-values caused by the geminal *O*-acetyl groups. The signals of H-6 and H-6′ were assigned by irradiating the signal of H-5. Although the n.m.r. spectrum of **4** was more complicated, assignments were made in comparison with the acetylated derivative (**3**), assuming that both spectra would be similar except for the deshielding effect of the three acetyl groups. The H-2 signal was identified as a doublet of doublets at highest field (near τ 6.2) from the $J_{1,2}$ value. N.m.r. parameters for all methylene and methine protons in **3** and **4** are listed in Table I. It is characteristic that the difference in chemical shift of H-2 between **3** and **4** is extremely large (1.51 p.p.m.) as compared with corresponding values for H-4 or H-5 (0.8–0.9 p.p.m.).

On comparing the chemical shifts of protons in the dimethyl acetals (**3** and **4**) with those in the corresponding α-pyranosides (**1** and **2**), moderate upfield shifts (0.2–0.4 p.p.m.) are observed for most protons, except for H-2 and H-5 in the acetylated derivatives. In contrast, comparison of coupling constants between **1** and **3**, or **2** and **4**, reveals noteworthy differences. The largest differences are seen in $J_{1,2}$ and $J_{3,4}$, which are increased by 4 to 5 Hz due to the opening of the pyranoid ring. At the same time, $J_{5,6}$ and $J_{5,6'}$ are increased by 1 to 2 Hz, whereas $J_{2,3}$ is decreased by 1.4 or 2.7 Hz.

In order to clarify the conformational significance of the coupling data observed, dihedral angles (ϕ) between all of the vicinal ring protons for compounds **1** and **2** were estimated from *J* values by applying the Karplus equation, as modified by Coxon[8] (Table II). It may be seen from the Table that the approximate *1C*(D) conformation of the pyranoid compounds **1** and **2** is extensively distorted from the idealized form, mainly through narrowing of the angle between H-2 and H-3 ($\phi_{2,3}$). This might be caused by C-2's moving away from the 3,6-anhydro ring to decrease the repulsion between H-1 and H-6. Recently Birch *et al.*[9] have also observed a similar deformation of the ring in 3,6:3′,6′-dianhydro-α,α-trehalose *1C*(D) tetrabenzoate.

TABLE II

APPROXIMATE PROTON–PROTON DIHEDRAL ANGLES (ϕ, DEGREES) CALCULATED FROM COUPLING CONSTANTS FOR DERIVATIVES OF 3,6-ANHYDRO-D-GALACTOSE

Compound	*Angles*					
	$\phi_{1,2}$	$\phi_{2,3}$	$\phi_{3,4}$	$\phi_{4,5}$	$\phi_{5,6}$	$\phi_{5,6'}$
1	51	37	71–78	59	71–78	52
2	52	32	71–78	58	71–78	51

Concerning the shape of the 3,6-anhydro furanoid ring in **1** and **2**, a twist form having C-5 below and C-4 above the plane defined by C-3, O-3(6), and C-6 can be postulated, because both $\phi_{3,4}$ and $\phi_{5,6}$ are appreciably larger than 60°, whereas $\phi_{4,5}$ remains nearer 60°. However, the opening of the pyranoid ring appears to cause extensive alteration in the shape of the furanoid ring, because $\phi_{3,4}$ and $\phi_{5,6'}$ in the two dimethyl acetals (**3** and **4**) may be presumed to be considerably different from those in **1** and **2**, as seen from the differences in coupling constants (Table I). Although $J_{1,2}$ values for **3** and **4** (7.3 and 6.6 Hz, respectively) indicate that rotation about the C-1–C-2 bond occurs quite freely, the unexpectedly small values of $J_{2,3}$ (3.7 and 3.1 Hz) suggest that rotamers in which H-2 and H-3 are gauche make a greater contribution than the rotamer in which these protons are antiparallel.

All of the foregoing observations may prove useful in the structural analysis of polysaccharides from red seaweeds. For instance, it is evident that the 3,6-anhydro ring involved in any mixture of sugars can be easily detected by the measurement of specific signals. The conformations of whole polysaccharide macromolecules might also possibly be deduced from those of the constituent furanoid and pyranoid residues detected by the analysis of n.m.r. data.

EXPERIMENTAL

N.m.r. spectra were measured at 100 MHz, in the constant field–frequency-sweep mode, with a JNM-4H-100 spectrometer, for 5–10% sugar solutions. Frequency-swept, double-resonance experiments were performed with an external, audiooscillator for the purpose of spin-decoupling. Sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) (in deuterium oxide) or hexamethyldisiloxane (HMDS) (in chloroform-*d*) was used as the lock signal and the internal standard. The temperature in the probe was 20°. Chemical shifts are expressed on the τ scale (DSS 9.98, HMDS 9.95) and coupling constants (J) are given in Hz.

A synthetic sample[10] of methyl 3,6-anhydro-α-D-galactopyranoside (**2**) was kindly provided by Dr. S. Hirase, Kyoto Technical University. Methyl 2,4-di-*O*-acetyl-3,6-anhydro-α-D-galactopyranoside (**1**) was prepared from **2** by acetylating it with acetic anhydride–pyridine. A solution of **2** (100 mg) in acetic anhydride (1.0 ml) containing anhydrous pyridine (0.5 ml) was kept for 2 h at 70°, and evaporated below

40° to give **1** as a faintly yellow syrup, which was analyzed by n.m.r. spectroscopy and identified by comparing the signal areas of the seven ring protons with those of the protons in the methoxyl group (τ 6.52) and in the two acetyl groups (τ 7.86, 7.94). 3,6-Anhydro-D-galactose dimethyl acetal (**4**) was also prepared from **2** according to the method described by Araki[11]. 2,4,5-Tri-*O*-acetyl-3,6-anhydro-D-galactose dimethyl acetal (**3**) was prepared from **4** by the acetylation procedure used for **2**. The product, a faintly yellow syrup, was analyzed by n.m.r. spectroscopy, and identified by comparing the signal areas of the protons in the two methoxyl groups (τ 6.62, 6.68) and in the three acetyl groups (τ 7.89, 7.94, 7.94) with those of the other seven protons.

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