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The effect of anomerism and hydration on the C–O–S vibrational frequency of D-galactose-3-sulfate determined by FTIR spectroscopy [1]

Kalavelil M. Koshy [a], Joan M. Boggs [a,b,*]

[a] *Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8*
[b] *Department of Clinical Biochemistry, University of Toronto, Toronto, Ontario, Canada M5G 1L5*

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Abstract

The effect of the anomeric structure of D-galactose-3-sulfate (potassium salt) on its spectral characteristics was studied by ^{1}H and ^{13}C NMR and FTIR spectroscopy under a variety of conditions. The synthetic product in D_2O was a mixture of α and β galactopyranose-3-sulfate. Crystallization from 80% ethanol yielded the α anomer in a high state of purity. The FTIR spectrum of the C–O–S vibrational bands in the region 900–800 cm^{-1} depended on the anomeric structure and on hydration. In the dry state the α anomer had a band at 868 cm^{-1}, while the β anomer had bands at 882 and 831 cm^{-1}. In D_2O the band at 868 cm^{-1} shifted down to 849 cm^{-1} for the α anomer and that at 831 cm^{-1} shifted down to 821 cm^{-1} for the β anomer. These differences in frequency will be useful as a diagnostic tool in determining the configuration at C-1 of galactose-3-sulfate units present in many molecules of biological importance. © 1997 Elsevier Science Ltd.

Keywords: Galactose-3-sulfate; Anomeric structure; Cerebroside sulfate; C–O–S vibrational frequency

1. Introduction

Sulfated saccharides are present in many biologically important molecules such as glycoproteins and glycolipids [1,2]. Galactose-3-sulfate is present as the hydrophilic head group in cerebroside sulfate, which is enriched in myelin [3], and in sulfogalactosyldiglyceride found in sperm [4], and as part of the oligosaccharide of a proteoglycan from some marine sponges [5]. A number of important biological processes, such as cell–cell recognition and adhesion, may occur partly through divalent cation-mediated carbohydrate–carbohydrate interactions involving a variety of sugars, including galactose-3-sulfate [6,7]. Divalent cations cause aggregation of liposomes containing galactosylceramide with liposomes containing cerebroside sulfate [8] and complexation of monomers

* Corresponding author. Biochemistry Department, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8. Tel.: +1-416-8135919; fax: +1-416-8135022.

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of these two glycolipids in methanol [9] suggesting that they form a ternary complex with the sugar head groups of these lipids, galactose, and galactose-3-sulfate.

In the course of an investigation the interaction between the carbohydrate head groups of cerebroside sulfate and galactosylceramide, we examined the FTIR spectra of synthetic galactose-3-sulfate (potassium salt) under a variety of conditions with emphasis on the C–O–S vibrational frequency in the spectral region 900–800 cm^{-1}. Because of the fact that some of the infrared absorption frequencies of monosaccharide sulfates, especially the C–O–S vibrational mode, appear to be highly characteristic of their structures, attempts have been made in the past to correlate these frequencies with the structures [10,11]. However, no consistent patterns have emerged [12], due in part to the many factors that influence the absorption frequencies in any given structure and the lack of definitive methods of structure verification. We show here that the anomeric configuration at C-1 of the sugar ring and the degree of hydration are two previously unrecognized factors which can significantly influence this frequency in the spectral region between 900 and 800 cm^{-1}.

2. Materials and methods

All chemicals and solvents used were analytical grade or of comparable quality and were used without further purification. D-Galactose was purchased from Sigma Chemical Co. (St. Louis, MO, USA). D_2O was from Merck, Sharp, and Dohme (Montreal, Canada), Me_2SO-d_6 was from Sigma, and KBr was from Wilmad Glass Co. (NJ, USA). The potassium salt of galactose-3-sulfate was synthesized by the method described by Archibald et al. [13]. Bovine brain galactosylceramide I[3]-sulfate (CBS) was isolated from a crude cerebroside fraction of bovine brain lipids (supplied by Avanti Polar Lipids, Birmingham, AL) by a modification of the method of Svennerholm and Thorin [14].

^{13}C NMR spectra were recorded on a Bruker AM 300 NMR spectrometer and ^{3}H NMR spectra on a Varian Unity Plus 500 MHz spectrometer at the NMR center, University of Toronto, Canada. Infrared spectra were run on a Bruker 1FS48 FTIR spectrometer equipped with a germanium-coated KBr beam splitter and a DTGS detector. The nominal spectral resolution was 2 cm^{-1}. For solid-phase spectra, normally 1 mg of the sample was mixed thoroughly with about 75 mg of powdered KBr and pressed into a 7 mm diameter pellet with a KBr Quick Press (Wilmad). For solution spectra, the samples were lyophilized from D_2O three times and dissolved in D_2O to give the desired concentration (usually 10% w/v, unless otherwise specified). The solution was placed in a standard IR cell assembly consisting of two 13 mm ZnSe windows and a 0.025 mm Teflon spacer. D_2O reference spectra were run concurrently under identical conditions. Evaporated films of the sugars were prepared by dissolving 1 mg of the sample in 50 μL of H_2O, placing the solution on a ZnSe window and allowing the water to evaporate in a desiccator over silica gel for 24 h. CBS was measured as a KBr pellet. Spectra were scanned from 4000 to 600 cm^{-1} when using a ZnSe window or from 4000 to 400 cm^{-1} when using KBr pellets. For solid-phase spectra, 100 scans, and for solution spectra, 250 scans were coadded and apodized with a triangular function. In the former case, the resulting spectra were then smoothed by a Savitzki–Golay function. For solution spectra, similar smoothing was done after subtraction of a D_2O spectrum. Appropriate baseline correction was applied in all cases. For time-dependent spectra, the time was noted when the spectrometer completed half the preset number of scans. The spectral data were processed using standard commercially available software.

3. Results and discussion

Characterization of synthetic galactose-3-sulfate. —^{13}C NMR spectroscopy has been shown [13,15] to be the method of choice for the unequivocal identification of the structure of various hexose monosulfates. Comparison of the ^{13}C NMR chemical shift values of galactose-3-sulfate synthesized by the method of Archibald et al. [13] with their reported values for the two anomeric forms of this sugar showed that in D_2O it was mainly a mixture of α and β galactopyranose-3-sulfate. All ^{13}C chemical shifts agreed with the literature data within the limits of experimental error. At the concentration used to record the spectrum (about 0.5 M), the ratio of α to β anomers was approximately 4:6, as estimated from the average ratio of peak heights of the resonances due to the α and β forms in the ^{13}C NMR spectrum. The identity and structure of the compound was further confirmed by ^{3}H NMR spectroscopy in D_2O. The values of the chemical shift and coupling constant of H-1 of resonances due to the two anomers

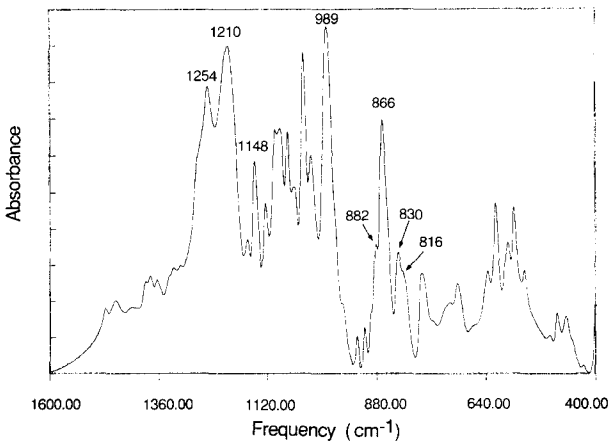

Fig. 1. FTIR spectrum of a dry sample of galactose-3-(potassium sulfate) in the region 1600–400 cm^{-1} showing the main features characteristic of a sugar sulfate (KBr pellet).

(see Table 1) were typical of those found in similar structures [16]. Consistent with the larger dihedral angle between the H and OH groups on C-1 in the β form compared to the α form, the observed value of the coupling constant $J_{H\text{-}1,H\text{-}1'}$ is larger in the β form than in the α form. For similar reasons, the $J_{H\text{-}1,H\text{-}2}$ value is also higher for the β anomer compared to the α anomer. The relative values of the chemical shifts of the anomeric protons in the two forms follow the same order as has been found for the parent hexose [17], i.e., the α anomeric proton resonates noticeably downfield from the β.

The FTIR spectrum of the compound in KBr showed bands characteristic of the S=O asymmetric stretch with maxima at 1254 and 1210 cm^{-1}, C–O stretch at 1148 cm^{-1}, a strong band at 989 cm^{-1} usually attributed to the sulfate ester-linked C–O stretch [18], and the C–O–S vibration in the region 900–800 cm^{-1} (Fig. 1). The two major bands observed in the 900–800 cm^{-1} region, after preliminary drying of the sample, had maxima at 863 and 820 cm^{-1} (not shown), in good agreement with the values reported in the literature [13]. However, as will be discussed later, after prolonged drying, as for Fig. 1, the C–O–S vibration, which is generally considered to be a combination of C–O stretch and C–O–S bending, gave a band at 866 cm^{-1}, with bands of lower intensity on either side at 882 and 830 cm^{-1}, and a shoulder at 816 cm^{-1}.

Isolation of α-D-galactopyranose-3-sulfate and its spectral characteristics.—By allowing D-galactose-3-sulfate (potassium salt) to crystallize slowly from 80% ethanol, it was possible to obtain one anomer in a high state of purity. The FTIR spectrum of the product in Kbr showed only a single strong band at 868 cm^{-1} in the C–O–S region (Fig. 2A). Except for vestiges of the other peaks which are present in the spectrum of the original mixture (Fig. 1) viz. at 882 and 831 cm^{-1}, there were no other prominent features in this region, consistent with the preponderance of one anomeric form. The ^1H NMR spectrum of the crystals dissolved in Me_2SO-d_6 showed approximately 85% of a major component and 15% of a minor component. The major component was determined to be the α anomer and the minor component was the β anomer from the coupling constants and chemical shift values (Table 1) [13,15,19]. The parent hexose also crystallizes from ethanol as the α anomer [22]. Angyal has shown [23] that interconversion between anomeric forms occurs slowly even in Me_2SO solution in the case of reducing sugars. A time lag of 2–3 days between dissolution of the crystals and the NMR measurements thus explains the presence of some β anomer in solution. The amount is considerably less than that found for the unresolved mixture in the same solvent.

Table 1
^1H NMR parameters for the H and OH groups at C-1 of the pyranose ring in α- and β-D-galactose-3-(potassium sulfate) [a]

	H (in Me_2SO-d_6)		OH (in Me_2SO-d_6)		H (in D_2O)	
	δ (ppm) (from Me_4Si)	$J_{H\text{-}1,H\text{-}2}$ (Hz)	δ (ppm) (from Me_4Si)	$J_{H\text{-}1,H\text{-}1'}$ (Hz)	δ ppm (from Me_4Si)	$J_{H\text{-}1,H\text{-}2}$ (Hz)
α-D-Galactose-3-(potassium sulfate)	4.911	3.84	6.166 (6.10)	4.39 (4.55) [a]	5.352 (5.23)	3.91 (2.7)
β-D-Galactose-3-(potassium sulfate)	4.255	7.51	6.517 (6.49) [a]	7.14 (6.65) [a]	4.717 (4.57) [b]	7.93 (6.7) [b]

[a] Numbers in parentheses represent the values found for the parent hexose from (a) ref. [20] and (b) ref. [17]. Although the chemical shift values of the OH are variable, their relative positions are useful in obtaining structural information [20,21].

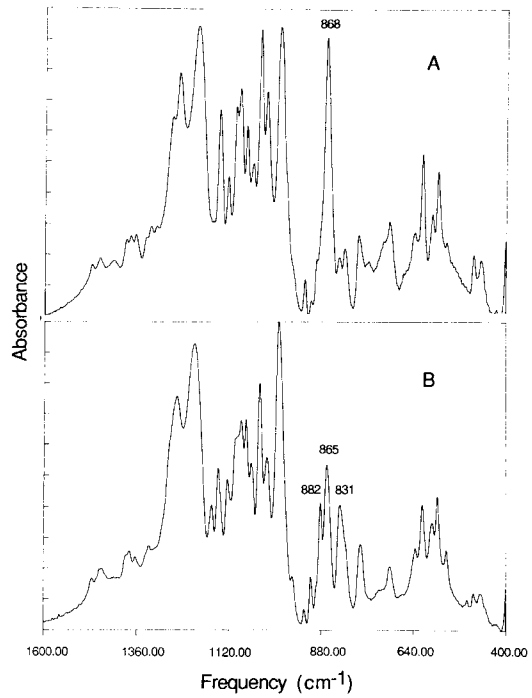

Fig. 2. FTIR spectrum in the region 1600–400 cm^{-1} of (A) α-D-galactose-3-sulfate. (B) After anomerization. (Both in Kbr pellets.)

Further support for the conclusion that the crystals consisted mainly of the α anomer came from the FTIR spectrum of galactosyl ceramide I^3-sulfate (from bovine brain) in which the galactose-3-sulfate is known to be in a β glycosidic linkage with the ceramide moiety [3]. The main absorption band in the C–O–S region of this lipid is at 825 cm^{-1} (Fig. 3). All the evidence discussed above thus supports the α-anomeric configuration for the component which crystallizes from 80% ethanol.

Effect of anomerization of α-D-*galactopyranose-3-sulfate on its FTIR spectrum.*—Establishment of the anomeric equilibrium in most simple sugars is relatively fast in aqueous solutions [24]. The rate of anomerization of unsubstituted α-D-galactose in D_2O solution was recently investigated by FTIR spectroscopy [25]. Consistent with earlier studies, the equilibrium was established in a few hours. In the case of galactose-3-sulfate, the rate of anomerization can e reasonably assumed to be similar to that of the parent hexose. After allowing a solution of α-D-galactose-3-sulfate in H_2O to establish equilibrium for about 16 h, the product was precipitated by the addition of ethanol. After washing the precipitate with ethanol and drying thoroughly, the FTIR spectrum was recorded. The spectrum obtained (Fig. 2B) is nearly identical with the spectrum of the unresolved α,β mixture in Fig. 1. By examining the C–O–S region of the spectrum it is clear that the 868 cm^{-1} band characteristic of the α anomer in the crystalline state has decreased considerably in intensity and slightly in frequency (to 865 cm^{-1}) and new bands are present at 882 and 831 cm^{-1} with a shoulder at the low frequency side. Since the latter two bands are present in the unresolved mixture (Fig. 1) and they reappear on anomerization of the α form, we conclude that they originate from the β anomer. Our experiments thus suggest that in the dry state an 868 cm^{-1} band is characteristic of the α anomer, while bands at 882 and 831 cm^{-1} are characteristic of the β anomer.

Thus the change in the conformation of the -H and -OH groups on C-1 of the sugar ring brings about a change in the frequency of the C–O–S stretching/bending modes on C-3. Since the sulfate group maintains the equatorial conformation in both structures, it is not easy to explain this difference on the basis of any direct interaction between the sulfate and -OH group on C-1, such as intramolecular hydrogen bonding or of steric interference. The role of interplay between the vibrational modes of the various groups in carbohydrate molecules in determining the absorption frequencies in the region between 900 and 700 cm^{-1} has been discussed by various authors ([26] and references therein). Although the origin of some of the bands in this region is far from clear, a number of useful correlations have emerged, allowing them to be used for distinguishing between α and β anomeric forms. In a similar manner, the difference in C–O–S vibrational frequency between the two anomeric configurations of D-galactose-3-sulfate will thus be useful as a diagnostic tool in determining the configuration at C-1 of galactose-sulfate units present in many molecules of biological importance.

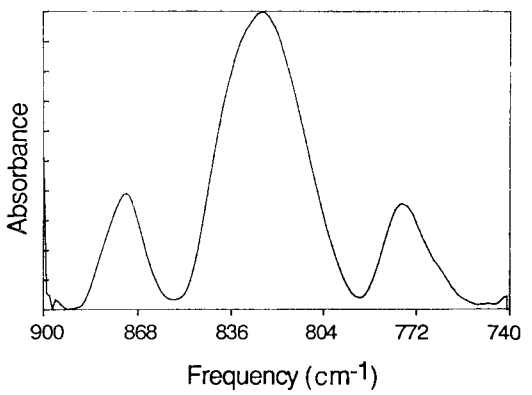

Fig. 3. FTIR spectrum in the region 900–740 cm^{-1} of bovine brain cerebroside sulfate (KBr pellet).

In order to gain a qualitative understanding of the rate at which the α-D-galactopyranose-3-sulfate undergoes anomerization, the decrease in intensity of the C–O–S vibrational frequency was followed with time in D_2O. As is evident from the spectra shown in Fig. 4, on dissolution of the α anomer in D_2O, the frequency shifts from 868 cm^{-1} down to 849 cm^{-1}. This shift is due to hydration of the α anomer. Exposure to moisture of the dry sample containing a mixture of anomers, used for Fig. 1, also caused a shift in frequency of the bands in this region as shown in Fig. 5B (compared to this region of the spectrum for the dry sample in Fig. 5A). The band at 866 cm^{-1} in the dry sample due to the α anomer (Fig. 2A) shifted to 852 cm^{-1} and the ban d at 830 cm^{-1} due to the β anomer shifted to 819 cm^{-1}. There are still shoulders at 865 and 882 cm^{-1}. The spectrum of this slightly hydrated sample resembles that of a film prepared by evaporation of an aqueous solution of the anomeric mixture in Fig. 5C. Fig. 5D shows the spectrum of a solution of the compound in D_2O. This fully hydrated sample has two main absorption bands with maxima at 849 and 821 cm^{-1}. Thus these frequencies must be characteristic of the hydrated α anomer and β anomer, respectively. This effect of hydration on the frequency of the C–O–S vibration has not previously been recognized and may account for some variations in frequencies reported for some sugar sulfates. The phase of the sample has previously been shown to have an effect on the spectrum of α-D-glucopyranose-2,3-disulfate (barium salt) [12] and sucrose [26]. The degree of hydration is another important factor that must be taken into account when using these frequencies for structural characterization.

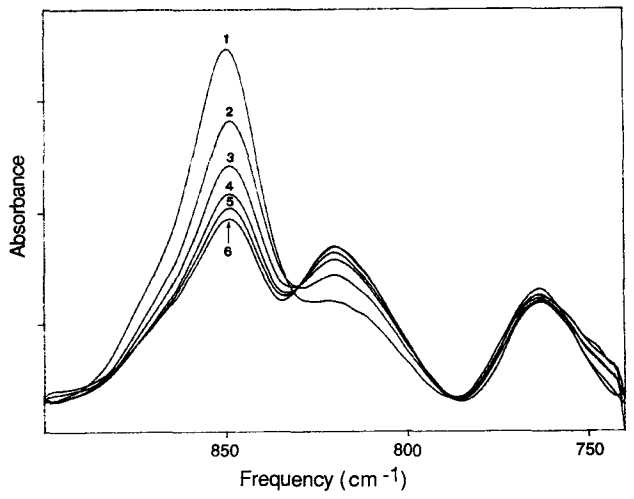

Fig. 4. FTIR spectrum of α-D-galactose-3-(potassium sulfate) measured at 20-min intervals beginning with 12 min (curve 1) after dissolution in D_2O, showing the decrease in the intensity of the peak at 849 cm^{-1} and the increase in that at 819 cm^{-1}.

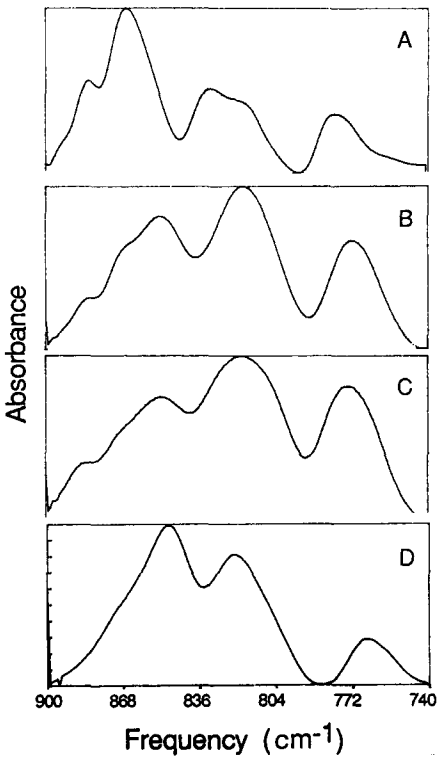

Fig. 5. FTIR spectrum of galactose-3-(potassium sulfate) in the region 900–740 cm^{-1} measured under various conditions. (A) Expansion of the above region from Fig. 1 of a thoroughly dry sample in KBr pellet. (B) After deliberate exposure of same sample to atmospheric moisture. (C) Evaporated film from H_2O solution. (D) 10% solution in D_2O.

With time, the spectrum of the aqueous solution of α-D-galactopyranose-3-sulfate showed a decrease in intensity of the peak at 849 cm^{-1} and a corresponding increase in the intensity of the band at 821 cm^{-1} due to an increase in the amount of the β anomer (Fig. 4). Equilibrium was nearly established at the end of two hours. Thus at ambient temperature, the rate of anomerization of galactose-3-sulfate is comparable to that of the parent sugar [25,27]. The conversion of α-D-galactose to the equilibrium mixture followed complex kinetics [24]. Thus no attempt was made in the present case to fit the data to any kinetic equation.

The effect of concentration on the anomeric equilibrium.—FTIR spectra were recorded of solutions of the anomeric mixture of galactose-3-sulfate (potassium salt) in D_2O at different concentrations from 5 to 15% (w/v) after allowing equilibrium to be

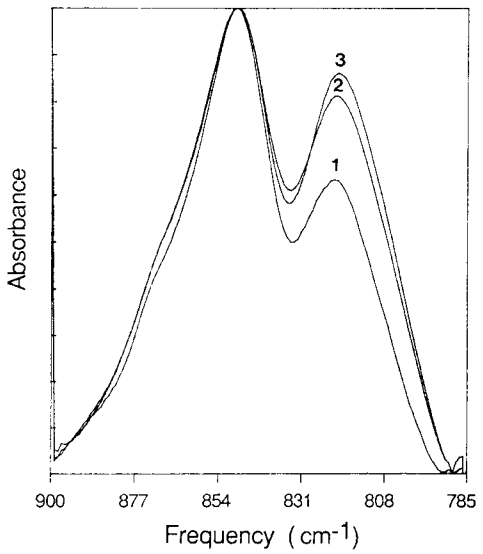

Fig. 6. The effect of changing the concentration of D-galactose-3-(potassium sulfate) on the relative intensities of the bands at 849 and 819 cm $^{-1}$. Curves 1, 2, and 3 represent concentrations of 5%, 10%, and 15% (w/v), respectively. All three spectra have been normalized to the intensity of the 849 cm $^{-1}$ peak in order to show the relative change in intensity of the 819 cm^{-1} peak.

reestablished. As is evident from the spectra shown in Fig. 6, there is a significant concentration dependence of the relative intensities of the peaks at 849 and 821 cm^{-1} even though the concentrations used are well below that at saturation. The relative intensity of the peak at 821 cm^{-1} increases with an increase in the total concentration of the sugar sulfate, suggesting that the degree of conversion to the β anomer at equilibrium increases with concentration. Thus the β anomer appears to be stabilized at higher concentrations. This could be caused by greater participation of the β anomer in intermolecularly hydrogen bonded aggregates at higher concentrations, which would shift the equilibrium to favour the β anomer. The equatorial conformation of the -OH group attached to C-1 in the β anomer would allow an edgewise approach of a second molecule. Furthermore, equatorial OH groups can participate as both hydrogen donors and acceptors, while axial OH groups can participate only as donors [23]. Equatorial OH groups thus form stronger intermolecular hydrogen bonds to water or other solvent molecules in solution or to other sugar molecules in crystals [23,28]. Although intermolecular hydrogen bonding between sugar molecules would be weakened, and could be eliminated in water, the fact that the β anomer, which is capable of greater intermolecular hydrogen bonding than the α anomer, is stabilized at higher concentrations, suggests that the β anomer may indeed participate in a greater degree of intermolecular hydrogen bonding with other sugar molecules in water as the sugar concentration increases. The concentration dependence of the anomer ratios of galactose-3-sulfate shown here may not be an isolated case, although evidence for it from studies of other sugars in the literature is limited. One example worth mentioning is that of glucose where a change in anomeric ratio was also observed with a change in concentration [29]. A concentration dependence may also explain why the reported ratio for the same sugar, as determined by ^{13}C NMR, varies in different studies, as noted by Angyal [23].

4. Conclusions

Although several empirical correlations between the C–O–S vibrational frequencies and structure have been proposed in the past, no study has addressed the question of the effect of anomerism on these bands. To our knowledge this is the first investigation of the effect of anomerism on the C–O–S vibration. Although the IR absorption frequency of this group in solid forms of methyl α-D-galactopyranose-4-sulfate (817 cm^{-1}) and benzyl β-D-galactopyranose-4-sulfate (853 cm^{-1}) has been reported by Harris and Turvey [12], the observed difference was attributed by them to the difference in the aglycone. In the light of our results, it would appear that this difference might be due to the difference in the anomeric structure. Although the order of the frequencies of these compounds, with the α anomer being less than the β anomer, is the opposite to that for galactose-3-sulfate, this may be due to the fact that the sulfate on C-4 is axial while that on C-3 is equatorial.

The C–O–S absorption frequency in sugar sulfates may be influenced by factors such as its position on the sugar ring [12,13]. We show that the anomeric structure is another important factor which needs to be taken into consideration. These frequencies can be used as a diagnostic tool for structure determination but this must be done with care. We have also shown that the degree of hydration of the sample has a significant effect on these frequencies. On the other hand, the sensitivity of these frequencies to structural and environmental effects may make them suitable for monitoring variations of these factors due to intermolecular interactions.

We also noted a dependence of the α to β anomeric ratio on galactose-3-sulfate concentration in

water. This concentration dependence suggests that the β anomer may be more involved in intermolecular hydrogen bonding with other sugar molecules than the α anomer.

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