

ASSESSMENT OF ^{13}C -SHIFT PARAMETERS IN DI- AND TRI-*O*- (3-NITROPROPANOYL)-D-GLUCOPYRANOSES

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ABSTRACT

Natural-abundance ^{13}C -n.m.r. spectra of seven di- and tri-*O*-(3-nitropropanoyl)- α - and - β -D-glucopyranoses are reported and discussed. Linear regression-analysis of the extracted ^{13}C -substituted parameters associated with esterification at various positions of the α - and β -glucose skeletons yielded two sets of substituent chemical-shift constants. The chemical shifts calculated from these generated parameters agreed well with the experimentally obtained values for each compound. Additivity of the substituent-induced shifts as a function of esterification site and substituent orientation is discussed. The structures of three previously uncharacterized di- and tri-esters were ascertained by using the parameters established.

INTRODUCTION

Although ^{13}C chemical shifts induced by substituents in linear and branched-chain hydrocarbons¹⁻⁵, cyclohexanes⁶, fused-ring hydrocarbons⁷, and aliphatic and aromatic monoalcohols⁸ have been extensively studied, little work has been done with substituted carbohydrates. Per-substituted glucopyranoses^{9,10} have been investigated and induced shifts of monosubstituted glucopyranoses have been dealt with in previous studies¹¹⁻¹⁴, but no systematic studies on specifically substituted pyranoses have been conducted.

In previous reports¹⁵⁻¹⁸ on naturally occurring glucopyranose esters, we and others characterized 7 di- and tri-*O*-(3-nitropropanoyl)- α - and - β -D-glucopyranoses, all having the $^4\text{C}_1$ conformation. In the present study, we made assignments for all resonances in ^{13}C spectra of these compounds and evaluated the effects of esterification on the resonances of carbon atoms at proximate sites in the glucopyranose rings.

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EXPERIMENTAL

^{13}C Spectra were recorded in the noise-decoupled mode on a JEOL FX-60Q Fourier-transform spectrometer* at 15.04 MHz. The spectra were recorded with 8K data points and 4000-Hz sweep widths. Chemical shifts were measured with an accuracy of ± 0.05 p.p.m.

Each sample was dissolved in acetone- d_6 (approximately 10–20 mg/40 μL) in a 1.8-mm micro tube. Reference to external tetramethylsilane was established prior to recording each spectrum. Selective proton-decoupling experiments were conducted with the dual ^1H , ^{13}C micro probe.

Compounds **2a**, **3a**, **4a**, **4b**, **5b**, and **6b** have been previously characterized^{15–18}. Compound **2b** was obtained by mutarotation of **2a** in water. The mutarotated mixture contained 58% **2a** and 42% **2b**. ^1H -Spectral data indicated that all derivatives are in the $^4\text{C}_1(\text{D})$ pyranose conformation.

^{13}C -Chemical-shift assignments of seven compounds were made, based on estimated α - and β -ester substituent-effects and selective, heteronuclear-decoupling experiments. The seven compounds were arranged into two anomeric series.

The resonances associated with C-1 and C-6 were readily assigned because of their unique shift-positions. The resonances associated with carbon atoms α - and β - to the site of esterification were identified in compounds **2a** and **2b** by the induced-isotope ^{13}C -shift observed¹⁹ in D_2O vs. H_2O . Verification of assignment of C-2 and C-4 in **2a** was made by use of single-frequency proton decoupling. Observation of the upfield shifts for positions β to esterified sites and downfield for positions α to esterified sites established the trends for resonance assignments of the remaining compounds. The positions of $\text{CH}_2\text{-NO}_2$ resonances were established by single-frequency proton decoupling. For each compound, six equations, each corresponding to one of the six resonances in the glucopyranose carbon skeleton, were derived. The sum of all ester substituent-parameters of a specific carbon atom was set equal to the shift difference in p.p.m. between the carbon resonance of the compound and the corresponding carbon resonance of the appropriate reference compound, either α - or β -glucose. Linear regression-analysis was used to solve for the unknown parameters.

RESULTS AND DISCUSSION

Chemical-shift assignments for α - and β -glucose and their 3-nitropropanoyl esters are assembled in Table I. Except for **2b**, each compound was determined as the pure anomer. When shifts could not be assigned directly by the anticipated effect of esterification, single-frequency proton-decoupling was performed to verify the identity. Such shifts are starred and the appropriate proton-decoupling position in p.p.m. from

* Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

TABLE I

OBSERVED ^{13}C CHEMICAL SHIFTS^a OF *O*-3-NITROPROPANOYL- α - AND - β -GLUCOPYRANOSES AND UNSUBSTITUTED α - AND β -GLUCOPYRANOSES^{b,c}

Compound Substitution pattern	Series A						Compound	Series B					
	C-1	C-2	C-3	C-4	C-5	C-6		C-1	C-2	C-3	C-4	C-5	C-6
1a R ¹ , R ² , R ³ , R ⁴ , R ⁵ = H	92.9	72.8	73.9	70.9	72.2	61.9	1b	97.0	75.3	77.0	70.7	76.8	61.9
2a^d R ² , R ⁵ = NP R ¹ , R ³ , R ⁴ = H	90.0	75.1* (4.7)	71.0	71.2* (3.5)	69.5	64.4	2b^d	95.1	76.4	74.1	70.1	74.8	64.3
3a R ² , R ³ , R ⁵ = NP R ¹ , R ⁴ = H	89.7	72.2	73.2* (5.5)	68.5	69.3	63.7	—	—	—	—	—	—	—
4a R ¹ , R ² , R ⁵ = NP R ³ , R ⁴ = H	89.2	72.1* (4.8)	70.4	71.9	69.5	63.0	4b	92.5	73.6* (4.9)	74.3	70.0	75.1	63.8
5a R ¹ , R ⁵ = NP R ² , R ³ , R ⁴ = H	—	—	—	—	—	—	5b	95.2	73.0	76.9	70.3	75.1	64.2
6a R ¹ , R ⁴ , R ⁵ = NP R ² , R ³ = H	—	—	—	—	—	—	6b	94.8	73.0* (3.5)	74.2	71.4* (4.9)	72.5	63.0

^aChemical shifts (δ) are reported in p.p.m. relative to external Me₄Si. All spectra were obtained in acetone-*d*₆ except for α - and β -glucose, whose spectra were examined in various mixtures of acetone-*d*₆-H₂O from 1:4 to 4:1. Shifts were extrapolated to 100% acetone-*d*₆. *Chemical-shift assignments were verified by selective proton-decoupling; proton-irradiation positions are given in p.p.m. from internal Me₄Si. ^bThe resonances associated with the CH₂-NO₂ and CH₂-C(=O) groups were observed at approximately δ 70.1 and 31.0, respectively. ^cNP = 3-nitropropanoyl. ^dDetermined in an anomeric mixture containing 72% of **2a** and 28% **2b** in acetone-*d*₆.

internal tetramethylsilane is indicated in parentheses. Chemical shifts of all esters are reported in acetone- d_6 . Although α - and β -D-glucose are not soluble in abs. acetone, their respective chemical shifts in acetone could be obtained by gradual dilution of an aqueous solution with acetone until the solution composition was 4:1 acetone-water (saturation point). Shifts showed no change relative to external tetramethylsilane when extrapolated to 100% solution in acetone.

The substituent-induced chemical shifts ($\delta_{\text{NP}} - \delta_g$) observed*, namely, the difference between the resonance of a particular carbon atom in each ester derivative and the resonance of the equivalent carbon atom in the parent glucopyranose anomer are assembled in Table II. These values were used to derive a series of ester-sub-

TABLE II
SUBSTITUENT-INDUCED CHEMICAL SHIFTS^a (p.p.m.)

Compound	Subst. pattern	C-1	C-2	C-3	C-4	C-5	C-6
$(\delta_{\text{NP}} - \delta_{\alpha\text{-g}})^b$ Series A							
1a	—						
2a	2,6	-2.9	2.3	-2.9	0.3	-2.7	2.5
3a	2,3,6	-3.2	-0.6	-0.7	-2.4	-2.9	1.8
4a	1,2,6	-3.7	-0.7	-3.5	1.0	-2.7	1.1
$(\delta_{\text{NP}} - \delta_{\beta\text{-g}})^b$ Series B							
1b	—						
2b	2,6	-1.9	1.1	-2.9	-0.6	-2.0	2.4
4b	1,2,6	-4.5	-1.7	-2.7	-0.7	-1.7	1.9
5b	1,6	-1.8	-2.3	-0.1	-0.4	-1.7	2.3
6b	1,4,6	-2.2	-2.3	-2.8	0.7	-4.3	1.1

^aThe difference between the shift position of a particular carbon atom in any *O*-3-nitropropanoyl-D-glucopyranose and the resonance of the equivalent carbon atom in the parent glucopyranose, each referenced to external Me_4Si . ^bNP = 3-nitropropanoyl; $\alpha\text{-g}$ and $\beta\text{-g}$ = α - and β -glucose.

stituent shift-constants. Correlation of the expressions in Table III with the numerical values in Table II resulted in a set of 18 equations with ten unknowns (three compounds with ten constants) for series A and a set of 24 equations with seven unknowns (four compounds with seven constants) for series B. Each of the six glucopyranose nuclei of the 3-nitropropanoyl esters was expressed by means of equations represented by the summation of all α , β , and γ parameters that contributed to the ester-induced shift relative to glucose (Table III). Each equation was then set equal to the corresponding observed, substituent-induced, chemical shift ($\delta_{\text{NP}} - \delta_g$) (Table II). Linear regression-analyses of these parameters led to a solution that could be readily fitted to the experimental data. To obtain a best fit for series A, it was necessary to use three additional values to account for the effects of the axial ester found in compound

*NP = 3-nitropropanoyl.

TABLE III

BEST-FIT SUBSTITUENT-PARAMETERS OBTAINED BY LINEAR REGRESSION-ANALYSIS FOR REPLACEMENT OF OH BY $\text{NO}_2\text{CH}_2\text{CH}_2\text{C}(=\text{O})\text{O}$ (NP) IN α - AND β -GLUCOPYRANOSIDES^a

n-Substituted =

iso =

iso α_1 =

Series A		Series B	
n	iso	n	iso
—	$\alpha_1 = -0.6$	—	$\alpha_1 = -1.9$
$\alpha = 2.3$	$x = 2.2$	$\alpha = 2.1$	$\alpha = 1.0$
$\beta = -2.4$	$\beta = -2.7$	$\beta = -1.6$	$\beta = -2.4$
	$\gamma = 0.1$		$\gamma = -0.3$
	$\delta = -0.4$		$\delta = -0.1$
	iso^b		
	$\gamma_{1-3,5} = -0.2$		
	$\delta_{1-4} = 0.8$		
	$\delta_{1-6} = -1.2$		

^aThe α , β , γ , and δ designations indicate the position of the carbon atom in relation to the position of esterification. ^bThe number following 1- in the subscripts designate the carbon resonance undergoing the induced shift as a result of esterification of the axial 1-OH group.

TABLE IV

CORRELATION OF CALCULATED AND OBSERVED ^{13}C CHEMICAL SHIFTS OF *O*-3-NITROPROPANOYL-D-GLUCOPYRANOSE DERIVATIVES

Compound	C-1		C-2		C-3		C-4		C-5		C-6	
	Obs.	Calc.										
<i>Series A</i> δ (p.p.m.) ^a												
1a	92.9		72.8		73.9		70.9		72.2		61.9	
2a	90.0	89.8	75.1	75.0	71.0	70.8	71.2	71.1	69.5	69.4	64.4	64.2
3a	89.7	89.9	72.2	72.3	73.2	73.0	68.5	68.4	69.3	69.5	63.7	63.8
4a	89.2	89.2	72.1	72.3	70.4	70.6	71.9	71.9	69.5	69.2	63.0	63.0
<i>Series B</i> δ (p.p.m.) ^b												
1b	97.0		75.3		77.0		70.7		76.8		61.9	
2b	95.1	94.5	76.4	76.3	74.1	74.5	70.1	70.1	74.8	75.1	64.3	64.0
4b	92.5	92.6	73.6	73.9	74.3	74.2	70.0	70.0	75.1	74.8	63.8	63.9
5b	95.2	95.0	73.0	72.9	76.9	76.6	70.3	70.3	75.1	74.9	64.2	63.9
6b	94.8	94.9	73.0	72.6	74.2	74.2	71.4	71.3	72.5	72.5	63.0	63.6

^aAverage difference = 0.14 p.p.m., SD = 0.08, SE = 0.02, range in errors is 0–0.3 p.p.m. ^bAverage difference = 0.20 p.p.m., SD = 0.18, SE = 0.04, range in errors is 0–0.6 p.p.m.

4a. As all esterified hydroxyl groups of compounds in series B are equatorial, only seven parameters were necessary. The calculated shifts for both series A and B agree well with the observed values, differing on the average by 0.14 and 0.20 p.p.m., respectively (Table IV).

Unlike the other secondary ring-carbon atoms, the highly electronegatively substituted C-1-OH carbon atoms are not strongly deshielded by the directly bound ester. Instead, the resonances of the α - and β -anomeric carbon atoms are shifted upfield by 0.3 and 1.9 p.p.m., respectively. This example of carbonyl-group shielding is exceptional, as earlier studies have indicated that, in both aliphatic and alicyclic structures⁸, all esterified OH carbon atoms resonate downfield with respect to their alcohol precursors. Presumably, the ring oxygen atom perturbs the carbonyl group of the C-1 esters, forcing it into a shielding orientation with respect to C-1. The stereochemistry at the anomeric center obviously strongly influences the induced shifts of all other esterified C-OH resonances as well. In the α anomeric series, iso $\alpha = 2.2$, whereas in the β anomeric series, iso $\alpha = 1.0$. Likewise, C-5 resonances adjacent to esterified C-6-OH are for α anomers $n\beta = -2.4$ and β anomers, $n\beta = -1.6$ (Table III). These differences may be ascribed to intramolecular, hydrogen-bonding differences of both C-1-OH and C-1-O-(3-nitropropanoyl) in the respective anomeric configurations. A similar observation concerning the differences in induced shifts of directly esterified C-OH resonances has been made in a study of 3-monoesterified α - and β -glucopyranoses¹³. The largest calculated shift-error noted in series B was found for C-6 of compound **6b**, which has the 1,4,6- β -substitution pattern. The underestimation of the observed high-field position of the C-6 resonance is, we consider, a result of not taking into account shielding by the carbonyl group at C-4. This shielding effect has been documented in our recent 220-MHz ¹H study of this compound¹⁸.

Compound **4a** is the only member of series A containing an axial ester group. From the data for **4a**, we observed that ester groups appear to exert diverse shifting-effects on the resonances associated with remote carbon atoms. For example, resonances of C-4 and C-6 (both *p* to the axial ester at C-1) undergo shifts of 0.8 and -1.2 p.p.m., respectively, whereas the resonances of C-3 and C-5 (both δ to the ester at C-1) are shielded by -0.2 p.p.m. Although it is common to observe shielding in the δ positions, deshielding in the γ positions of esterified hydroxyl compounds has been documented only in aromatic derivatives⁸.

To test the validity of the parameters given in Table III, we calculated the chemical shifts of three, previously uncharacterized 3-*O*-nitropropanoyl-D-glucopyranoses. Aside from the determination that compounds **7b** and **8a** were bis- and tris-(3-nitropropanoic) esters of glucose, respectively, the positions of substitution had not been established. The parameters in Table III were used to generate spectral shifts for various substituted di- and tri-esters. The best fit between calculated and empirical spectral-data for **7b**, **8a**, and **8b** (obtained in 2:3 mixture with **8a** by mutarotation of **8a**) was obtained by use of the assignments for 1,3,6-tri-*O*-(3-nitropropanoyl)- β -D-glucopyranose, 4,6-di-*O*-(3-nitropropanoyl)- α -D-glucopyranose, and 4,6-di-*O*-(3-nitropropanoyl)- β -D-glucopyranose, respectively, as a basis for calculation. The

TABLE V

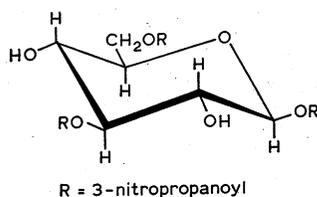
COMPARISON OF CALCULATED AND OBSERVED SPECTRAL SHIFTS FOR COMPOUNDS **7a**, **8a**, AND **8b**^a

Proposed structural assignment	Shifts, δ (p.p.m.)											
	C-1		C-2		C-3		C-4		C-5		C-6	
	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
1,3,6- β 7b	94.3	94.7	70.5	70.5	77.8	77.6	67.7	67.9	74.2	74.6	63.2	63.8
Δ		-0.4		0.0		0.2		-0.2		-0.4		-0.6
4,6- α 8a	92.6	92.1	72.2	72.9	71.3	70.8	72.7	73.2	67.2	67.1	63.6	64.3
Δ		0.5		-0.7		0.5		-0.5		0.1		-0.7
4,6- β 8b	97.2	96.8	75.3	75.0	74.3	74.5	71.5	71.4	72.1	72.8	63.6	63.7
Δ		-0.4		0.3		-0.2		0.1		0.7		0.1

^aAverage error for **7b** = 0.30, SD = 0.21, SE = 0.09; average error for **8a** = 0.48, SD = 0.26, SE = 0.11; average error for **8b** = 0.27, SD = 0.22, SE = 0.04.

average shift-errors (an average of the differences between calculated and observed values) were 0.30 (**7b**), 0.48 (**8a**), and 0.28 p.p.m. (**8b**) (Table V).

A typical calculation of shift positions is illustrated for compound **7b**, assuming the 1,3,6- β substitution pattern, as illustrated.



Carbon parameter used	Parameter sum (p.p.m.)	δ Calc. ($\delta_g + \text{Parameter sum}$)
C-1	iso $\alpha_1 + \gamma + \delta$	94.7
C-2	iso $\beta + \text{iso } \beta$	70.5
C-3	iso $\alpha + \gamma + \delta$	77.6
C-4	iso $\beta + \gamma + \delta$	67.9
C-5	n $\beta + \gamma + \gamma$	74.6
C-6	n $\alpha + \delta + \delta$	63.8

Alternative structures for compounds **7b**, **8a**, and **8b** were eliminated from consideration, because the average error associated with them exceeded those just cited by a factor of at least three. For example, average errors found for calculated shifts relative to empirically observed shifts in **7b** for the remaining possible alternative structures having the substitution patterns 1,4,6- α , 1,3,6- α , and 1,2,6- β , were 2.7, 3.2, and 1.7 p.p.m., respectively. Further examination of compounds **7b**, **8a**, and **8b** by use of 220-MHz, ¹H-n.m.r. spectra has subsequently corroborated the ¹³C assignments.

Although the results of this study appear internally consistent, it is difficult to assess what role the nitro group plays in inducing chemical shifts on the ring-carbon atoms. Presumably the effect of this group on induced shifts is minimal, because of its remote position from the carbonyl group. It is anticipated that substituent-shift parameters obtained from these model compounds may serve as a universal set to predict ester-induced chemical shifts or shift trends for many new positionally esterified pyranoid carbohydrates.

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