Nuclear Magnetic Resonance Characterization of Reaction Products of Interesterification of Peracetylated α-D-Glucopyranose and Fatty Acid Methyl Esters

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ABSTRACT: Mono- and diesters of fatty acids of peracetylated α-D-glucopyranose were prepared by chemical interesterification. Substituent-induced chemical shift effects on the carbonyl carbons rather than the ring carbons and proton atoms unambiguously show the fatty acyl substituents to be at C1 in the monosubstituted, and at C1 and C6 in the disubstituted products. ^{1}H nuclear magnetic resonance (NMR) integration data before and after interesterification complemented ^{13}C chemical shift data in verifying the molecular structures. Empirical data from classical ^{1}H and ^{13}C NMR experiments thus provide a simple self-contained method for determining the number and position of fatty acyl substituents, and the anomeric compositions of peracetylated glucose fatty esters.

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KEY WORDS: Interesterification, nuclear magnetic resonance, peracetylated glucose fatty esters, substituent-induced chemical shift.

Carbohydrate fatty esters, generally regarded as safe and environmentally friendly, have become the surfactant of choice in food, pharmaceutical, detergent, and cosmetic products. The development of high-resolution nuclear magnetic resonance (NMR) spectroscopy has enabled extensive tabulation of ^{13}C chemical shift data on the oligosaccharide ring systems, their methoxides, and acetates (1). Assignment of substituent groups on the ring is based on the presence or absence of OH proton signals in the hydrolyzed acylated products (2). In most cases, however, OH proton signals are unresolved and submerged in those of the ring methine protons. Besides ^{1}H and ^{13}C enrichment methods (3), the other method of assignment is based on substituent-induced chemical shift (SCS) influence on the ring carbon and proton atoms, which involves extensive decoupling experiments (4). While the latter approach has been relatively successful with respect to pyranoses (3), it has failed when applied to polyacylated pyranoses (5). This is due to complex electronic and steric perturbations introduced by the acyl groups—the shift patterns do not show any systematic relationship to substitution positions—rendering the shift data diagnostically unuseful. This situation becomes more complicated in the case of interesterification where the acyl moieties are only interchanged. In a preliminary communication (Obaje, O.J., D. Kuang, G.C. Ee, and H. Suhaimi, unpublished data), we reported the $^{3}J_{C-H}$ heteronuclear multiple-bond correlation (HMBC)-assisted assignments of the carbonyl carbon atoms in peracetylated α-D-glucopyranose. We have synthesized a number of fatty acyl-substituted peracetylated α-D-glucopyranoses by interesterification. We report here the SCS effects on the ^{13}C signals of the carbonyl carbon atoms of the esters. This data, in combination with usual NMR methods, allowed for the determination of the number and position of fatty acyl substituents in the esters and their anomeric compositions.

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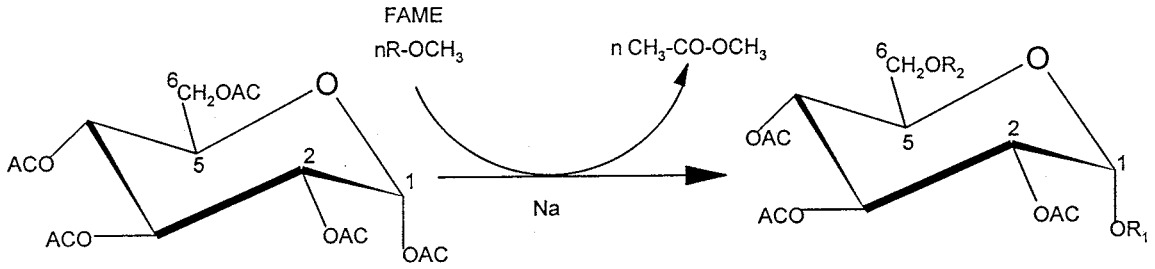
MATERIALS AND METHODS

Materials. α-D-Glucose pentaacetate (GPA) **1**, 99% purity) was purchased from Fluka Biochemika (Buchs, Switzerland). Fatty acid methyl esters (FAME) of oleic, stearic, palm, and palm kernel fatty acids were gifts from Henkel Oleochemicals (Kuala Langat, Malaysia). Silica gel 60 and all solvents (analytical grade) were obtained from E. Merck (Darmstadt, Germany).

Interesterification method. Mono- and di- fatty acid esters of acetylated glucopyranoses were prepared by interesterification reaction of GPA **1** with appropriate FAME in the presence of sodium metal catalyst, according to the method described by Akoh and Swanson (6), with minor modifications, and as shown in Scheme 1. Typically, FAME (15.1 mmol) was put in a three-necked, round-bottomed flask equipped with a magnetic stirrer, stopcocks, a vacuum take-off line leading to a liquid nitrogen cold trap, and a vacuum pump. The reaction flask was flushed with dry N_2 gas for 30 min before admixing the reactants. GPA (5.0 mmol) and sodium metal (0.12 g) were added, and heating commenced with continuous stirring. An oil bath was used to maintain the temperature between 80 and 100°C. The pressure was maintained at 0–25 mm Hg with a vacuum pump. The sodium metal catalyst and GPA melted and the reaction mixture became homogeneous after 30 min. The reaction was continued for 4 to 7 h. The product was neutralized with 1–3 mL glacial acetic



2. R_1 = Palm kernel fatty acyl moiety; R_2 = AC

3. $R_1 = R_2$ = Palm kernel fatty acyl moiety

4. R_1 = Palm fatty acyl moiety; R_2 = AC

5. $R_1 = R_2 =$ -CO-$(CH_2)_{4\text{-}8}$-CH_3

6. $R_1 =$ -CO-$(CH_2)_{16}$-CH_3; R_2 = AC

7. R_1 = -CO-$(CH_2)_7$-CH=CH-$(CH_2)_7CH_3$; R_2 = AC

SCHEME 1

acid, allowed to cool, dissolved in acetonitrile, and decolorized with 2 g activated charcoal.

The mono- and disubstituted glucose fatty acid esters were separated on a 40 × 2 cm silica gel column (packed with silica gel 60, particle size 0.063–0.200 mm; 70–230 mesh ASTM), and eluted with 20% ethanol and 10% ethyl acetate in hexane (vol/vol), respectively. The solvents were evaporated by rotary evaporation to give the corresponding products, which were mixtures of α and β anomers. Optical rotations were determined using a JASCO (Tokyo, Japan) DIP-370 digital polarimeter. In addition to NMR spectroscopy, the purity of compounds was confirmed by melting point and Fourier transform infrared experiments. A summary of the interesterification reaction conditions, yields, specific rotation, and anomeric compositions is given in Table 1.

NMR instrumentation. ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were recorded for 0.05–0.1 M $CDCl_3$ solutions at ambient temperatures (27–29°C) with a JEOL GX400 spectrometer (Tokyo, Japan), using acquisition times of 4.099 and 1.209 s, spectral widths of 4,000 and 13,550 Hz, respectively, and at 32 K data points each. Chemical shifts are given in ppm and referenced to tetramethylsilane (TMS) as an internal standard (δ = 0.00). The two-dimensional data were obtained using UNIX JEOL software.

RESULTS AND DISCUSSION

The ring carbon signals were assigned from the two-dimensional ^{1}H–^{13}C correlation spectroscopy (COSY) crosspeak contours as shown in Figure 1. The spectrum shows the correlation of the pyranosyl ring carbon atoms (δ_C 61.51 to 89.07 ppm) with the pyranosyl ring-proton atoms (δ_H 4.08 to 6.33 ppm). Ring protons in compound **1** were assigned according to those previously made by Utamura *et al.* (5). Seven distinct contours, arising from the five methine and two methylene protons, allowed for the assignment of the six-ring carbon atoms as given in Table 2.

The SCS effect on the ring protons, which are all δ-distance (3 bonds) to the carbonyl carbon, is minimal, varying

TABLE 1
Reaction Conditions[a] and Product Characteristics

Compound[b]	Max. temp. (°C)	Yield[c] (%)	α-Anomer[d] (%)	β-Anomer[d] (%)	$[\alpha]_D^{28}$ [e]
1 (GPA)	—	—	100	0	100.24 ± 2
2	91	66.6	81.9	18.1	41.86 ± 2
3	91	19.2	78.3	21.7	40.51 ± 2
4	89	91.4	82.4	17.6	51.70 ± 2
5	90	92.8	89.2	10.8	52.23 ± 2
6	91	70.6	90.2	9.8	53.51 ± 2
7	95	41.5	62.3	37.7	31.35 ± 2

[a]Mole ratio, α-D-glucose pentaacetate (GPA **1**)/fatty acid methyl esters (FAME **3**); reaction time, 6 h; and 0.12 g sodium metal catalyst.
[b]Molecular structures are given in Scheme 1.
[c]Percentage weight of product per theoretical weight based on the initial weight of GPA **1**.
[d]Calculated from the relative intensities of the corresponding anomeric proton signals in the ^{1}H nuclear magnetic resonance (NMR) spectrum (δH_α = 6.33 ppm; δH_β = 5.78 ppm).
[e]*c* = 1, methanol.

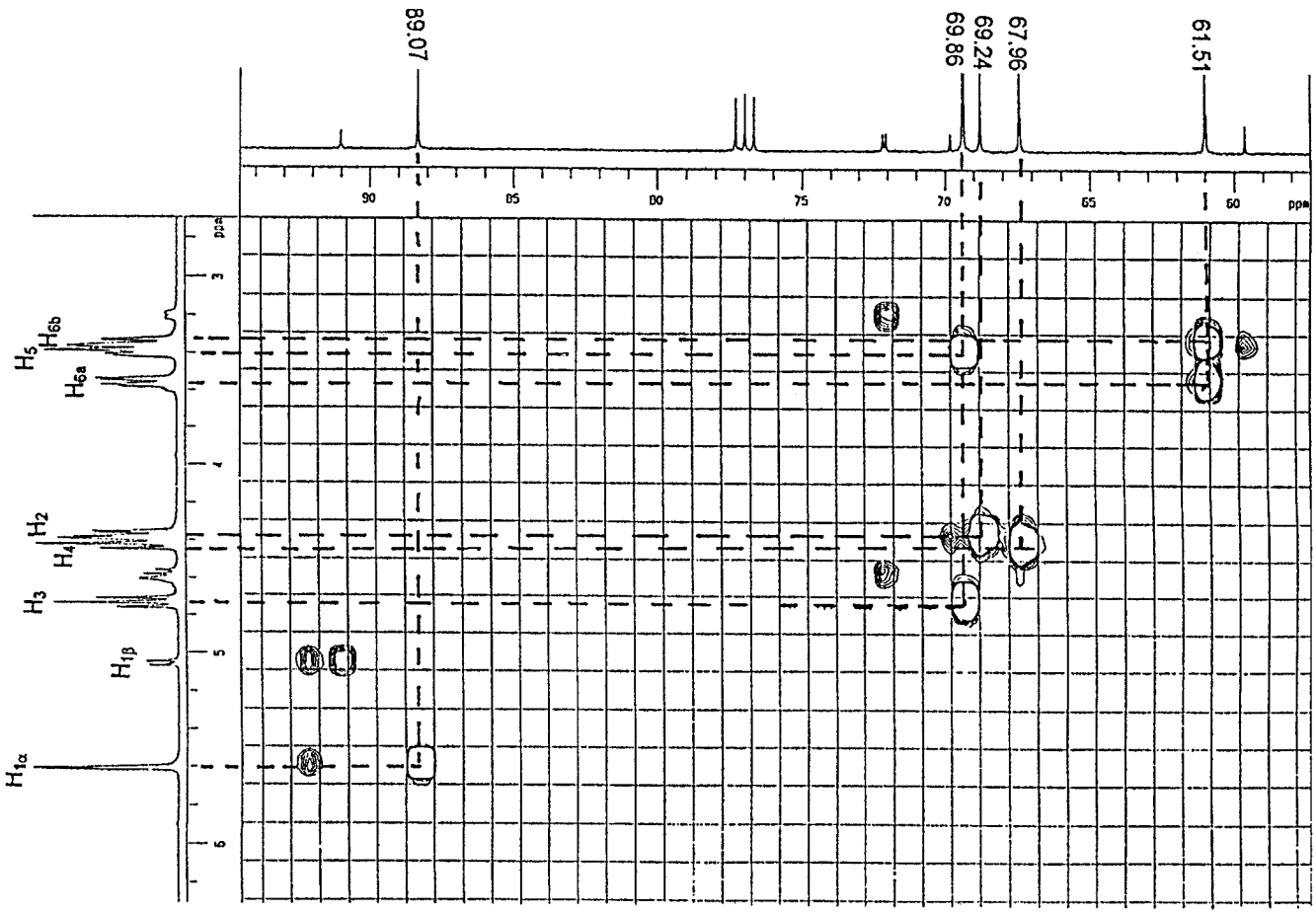


FIG. 1. Two-dimensional ^{1}H–^{13}C correlation spectrum of α-D-glucose pentaacetate, correlating pyranosyl ring protons with ring carbon atoms.

TABLE 2
Chemical Shift Differences on Interesterification, Relative to GPA 1[a]

		GPA **1**	$\Delta\delta^2$	$\Delta\delta^3$	$\Delta\delta^4$	$\Delta\delta^5$	$\Delta\delta^6$	$\Delta\delta^7$
Ring protons	H_1(*d*)	6.33	0	0.02	0.01	0	0.01	0
	H_2(*q*)	5.11	0.01	–0.01	–0.01	0.01	0.01	0.01
	H_3(*t*)	5.47	–0.02	0	–0.01	–0.02	0	–0.02
	H_4(*t*)	5.15	–0.01	–0.03	–0.03	–0.01	–0.01	0
	H_5(*o*)	4.11	0.01	–0.01	0	0.01	0.02	0
	H_{6a}(*q*)	4.27	0.01	0	0.01	0.01	0.02	0.04
	H_{6b}(*q*)	4.08	0	0	0.01	0	0.02	0
Ring carbon atoms	C_1	89.07	–0.13	–0.11	–0.09	–0.08	–0.19	–0.09
	C_2	69.24	0.17	0.21	0.20	0.21	0.10	0.18
	C_3	69.86	0.15	0.15	0.17	0.19	0.10	0.15
	C_4	67.96	0.11	0.14	0.13	0.15	0.03	0.11
	C_5	69.86	0.15	0.15	0.17	0.19	0.03	0.15
	C_6	61.51	0.14	0.14	0.14	0.19	0.02	0.14
Carbonyl carbon atoms	C_{a1}	170.53	0.88	1.66	0.93	1.65	1.03	0.83
	C_{a2}	169.38	0.07	0.61	0.10	0.09	0.17	0.01
	C_{a3}	169.62	0.34	0.82	0.44	0.74	0.53	0.29
	C_{a4}	168.73	0.58	0.63	0.34	0.63	0.65	0.21
	C_{a6}	170.15	0.19	1.40	0.28	1.42	0.39	0.14

[a]Ring protons in compound **1** were assigned according to those previously made in Reference 5. Signal multiplicities are symbolized by *d* = doublet; *t* = triplet; *o* = octet, and *q* = quartet. δ, ppm from tetramethylsilane (TMS) in $CDCl_3$. ($\Delta\delta^i = \delta^i_{product} - \delta_{reactant}$.) See Table 1 for abbreviation.

TABLE 3
Number of Protons by Type from Normalized ^{1}H NMR Integration Data[a]

Compound	Avg.mol wt. of FFA (g/mol)	Ring H $\delta_{H(4.10–6.33)}$	CH=CH $\delta_{H(5.33)}$	CH_2–CO $\delta_{H(2.40–2.44)}$	CH_3–COO $\delta_{H(1.98–2.20)}$	CH_2–CH_2CO $\delta_{H(1.59–1.68)}$	–$[CH_2]_n$– $\delta_{H(1.2–1.4)}$	Terminal –CH_3 $\delta_{H(0.88)}$	Total no. of protons obs.	Theoretical no. of protons
GPA **1**	—	7.07	—	—	15.57	—	—	—	22.64	22.00
2	229.1	6.28	1.83	1.83	13.31	1.83	17.97	3.00	46.05	46.70
3	229.1	7.56	*n*	4.62	10.18	4.36	43.18	6.00	75.90	74.40
4	269.0	6.66	1.81	2.26	12.00	2.18	25.91	3.71	53.63	53.53
5	159.2	8.30	—	3.57	13.05	3.93	18.42	6.00	53.27	54.00
6	284.2	5.54	*n*	*n*	12.00	1.80	32.68	3.22	55.24	54.00
7	282.2	5.19	2.00	2.00	14.51	2.00	21.65	3.47	50.82	52.00

[a]Observed integration data is the average of three successive integrals obtained for each sample at constant scan frequency and sweep time. Normalization of the integration data was done using the anomeric proton (δ = 6.33 ppm) and terminal methyl protons (δ = 0.88 ppm) as secondary references (TMS being the internal reference). δ, ppm from TMS in $CDCl_3$; *n* = unresolved signal; FFA, free fatty acid. For other abbreviations, see Tables 1 and 2.

from –0.03 ppm for $\Delta\delta^3_{H4}$ and $\Delta\delta^4_{H4}$ to 0.04 ppm for $\Delta\delta^7_{H6a}$ (Table 2). The origin of chemical shift differences in ^{1}H and ^{13}C signals are twofold; the through-bond inductive and the long-range, nonbonded through-space effects both result from the replacement of $–COCH_3$ with the fatty acyl group on interesterification. The observed chemical shift differences on the ring protons could be attributed to the combined inductive and nonbonded through-space SCS effects. These differences, however, do not reflect, empirically, the number and positions of fatty acid substituents. The ring anomeric carbon atoms (C1) do, however, show SCS changes, which are reflective of the fatty acid substitution at the anomeric carbon. The γ-upfield shift observed on all C1 for compounds **2–7** is in line with established steric SCS effect (7). Both inductive (γ_{gauche}) and the through-space (γ_{syn}) steric effect considerations (8) support the observed upfield shift on C1. The SCS effects on C6, however, fail to discriminate against the second fatty acid substituent in compounds **3** and **5**. This therefore limits the use of SCS effects on the ring carbon atoms for structural elucidation.

The α-deshielding (+ve difference) observed on C_{a1} and C_{a6} carbonyl carbons of compounds **2–7** is unambiguous. The observed SCS changes are not only significant but also consistent with respect to the positions of fatty acyl substituents. For instance, $\Delta\delta_{ca1}$ for compounds **2–7** are in the range 0.83 to 1.66 and are the highest in the series except for $\Delta\delta^3_{ca6}$ = 1.40 and $\Delta\delta^5_{ca6}$ = 1.42 attributed to the second fatty acyl substituent. The SCS changes on Ca1 and Ca6 clearly distinguish between the mono- and the diester fatty acyl substitution products. This is supported by the ^{1}H integration data in Table 3. Although deshielding influence is also observed on the other distant (≥5 bonds) carbonyl carbon atoms, it is less profound. While the former (α-SCS effect) is inductive in origin, the latter can only be explained in terms of a through-space nonbonded steric influence of the fatty acyl group. The steric influence may have caused further electron drift from C in C=O, leading to deshielding. The values of shift differences at C_{a3} and C_{a4} in both mono- and disubstituted products (i.e., compounds **3** and **5**) are, however, relatively higher compared with the other distant carbonyl carbon atoms. Although this may be attributable to conformational relationships between these carbon atoms, it cannot be concluded from the present data.

The ^{1}H integration data (Table 3) corroborate the number of fatty acyl substitutions in **2–7**. The differences between theoretical and observed protons vary from 0.65 in compound **2** to 1.5 in compound **3**. The largest difference represents only a 2.0% deviation from the theoretical value. This, in spite of the heterogeneous composition of the fatty acids involved, is impressive and provides a means to check on the results from SCS analysis. This method is therefore inherently self-contained. The relative effects of the fatty acyl groups on ^{13}C chemical shifts of carbonyl carbon atoms in peracetylated glucopyranoses thus provide empirical data for qualitative and structural characterization of their fatty esters. This offers a simple technique for characterizing interesterification products and for studying interesterification processes involving polyacylated oligo- and polysaccharides.

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