Journal of Oil Palm Research Vol. 12 No. 1, June 2000, p. 102.106

FATTY ACYL SUBSTITUENT-INDUCED CHANGES IN ^{13}C-CHEMICAL SHIFTS OF CARBONYL CARBONS:- A TOOL FOR STRUCTURAL ELUCIDATION IN ACETYLATED GLUCOSE ESTERS OF PALM FATTY ACID

Keywords: Acetylated glucose fatty ester, interesterification, polyacetylated glucopyranose, substituent-induced '%-chemical shift.

OLOBO J OBAJE*; DZULKEFLY KUANG*; HAMDAN SUHAIMI*; ANUAR KASSIM* and ZAIZI DESA*

* Chemistry Department, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

Substituent-induced changes (SIC) in ^{13}C-chemical shifts of carbonyl carbon atoms upon interesterification of glucose pentaacetate (GPA) with fatty acid methyl esters (FAME) of stripped palm kernel oil (SPKO), palm oil (PO) and cupric (C_{10}) acid, were used to determine the number and positions of the fatty acid substituents on the pyranosyl ring. Results show that SIC on the ring carbon and ringproton atoms are not always consistent with the molecularstructures. But SIC on thecarbonyl carbon atoms are however unambiguous and consistent with molecular structures of all the products. The analysis of ^{13}C-chemical shift changes of carbonyl carbons therefore provides a means to determine the number and positions of fatty acyl groups in polyacetylated glucopyranoses.

INTRODUCTION

Interesterification remains an economically preferred route for industrial production of carbohydrate fatty esters (Nakamura, 1997). A number of instrumental methods already exist which can distinguish the reactant esters from the product esters, depending on the chemical identities such as chain-length, unsaturation and other functional groups attached to the carbonyl carbon. If the product ester is, however, a polyester, such as in glycerides and glycosyl esters, assignment of the positions of substitution is not as straight forward. The wet chemistry analytical methods for achieving these objectives are tedious, time consuming and expensive. High-resolution NMR spectroscopy has been used to assign the positions of substituent groups on the pyranosyl ring based on the presence or absence of OH proton signals in the hydrolyzed acylated products (Khan *et al.*,

1996). In most cases however, OH proton signals are unresolved and submerged in those of the ring methine protons. The effect of esterification on the chemical shift of carbon atoms of alcohols has been studied by Terui and co-workers (1976). King-Morris and Serianni (1987) developed an empirical method which correlates pyranosyl ring configuration and conformation with ^{13}C chemical shifts by [^{13}C]-enrichment at the anomeric carbon. Their method of assignment included substituent-induced chemical shift on the ring carbon and proton atoms, involving extensive coupling and decoupling experiments. While this approach was relatively successful with respect to pyranoses, it failed in application to polyacetylated pyranoses (Utamura et al., 1986). Theintractability ofshift patterns in polyacetylated pyranoses is attributed to (i) complex electronic and steric perturbation introduced by the acyl groups, (ii) the bond distance of the acyl groups to the ring, and (iii) in the case of interesterification reactions, identical functional groups (only the acyl groups are interchanged between two esters such that both reactants and products remain esters).

This paper presents a simple empirical technique, based on the SIC in the ^{13}C-chemical shifts of the carbonyl-carbon atoms, which unequivocally identifies the number and positions of substitution of the fatty acyl group(s) in GPA.

EXPERIMENTAL

Materials and Method

Mono- and di-fatty acid substituted acetylated glucopyranoses were prepared by interesterification reaction of GPA with the appropriate FAME in the presence of Na-metal catalyst, according to the method described by Akoh and Swanson (1987), and modified by Obaje et al. (1999). GPA was obtained from Fluka Biochemika (Buchs, Switzerland) and the FAME of SPKO, PO and C_{10} acid were gifts from Henkel Oleochemicals (Banting, Malaysia). The reaction mixture of GPA, FAME (mole ratio of 1:3) and Na-metal catalyst (0.12 g) were heated at 85°C-90°C for about 6 hr, and at 20 mmHg. The products were separated and purified by column chromatography using a 40x2 cm 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 (v/v), respectively. Products were analyzed by TLC, FT-IR and confirmed by ^{1}H and ^{13}C NMR spectrometry.

FAME from the SPKO yielded 67% l-O-fatty acyl 2,3,4,6-acetyl a-D-glucopyranose (SPKO-1) and 21% 1,6-O-fatty acyl 2,3,4-acetyl a-D-glucopyranose (SPKO-2); FAME from PO yielded 91% l-O-fatty acyl 2,3,4,6-acetyl α-D-glucopyranose (PO-l); and C_{10} acid FAME, yielded 70% 1,6-O-fatty acyl 2,3,4-acetyl a-D-glucopyranose.

^{1}H and ^{13}C NMR Experiment

The ^{1}H-NMR spectra (400 MHz) and ^{13}C-NMR spectra (100 MHz) of interesterification products and reactant (glucose pentaacetate) were recorded for 0.05 M-0.1 M $CDCl_3$ solutions at ambient temperature (27±2°C) with a JEOL GX400 spectrometer, using acquisition times of 4.099 and 1.209 sec, spectral widths of 4000 and 13 550 Hz respectively, and at 32K data points each. Chemical shifts are given in ppm, referenced to tetramethylsilane (TMS) as internal standards (δ=0.00). The proton and the ring carbon chemical shifts were assigned by 2D ^{13}C-^{1}H shift correlation, and the carbonyl carbons were assigned by heteronuclear multiple bond correlation (HMBC).

The fatty acyl substituent-induced changes in chemical shifts ($\Delta\delta$) were calculated by subtracting the corresponding chemical shift of the reactant (GPA) from those of the products,

RESULTS AND DISCUSSION

The molecular structures ofthe products, SPKO-1, SPKO-2, PO-1 and C_{10}-2, are given in Figure *1:* The molecular structures were established by TLC, FT-IR, ^{1}H and ^{13}C NMR spectrometry, and are in conformity with those proposed by Bols (1996) and reported by Obaje et al. (1999).

Typical changes in the ^{13}C-chemical shift of the carbonyl carbons (δ_c 168-173 ppm) of SPKO-2 relative to GPA are given in Figure 2. The values of changes in ^{1}H- and ^{13}C-chemical shifts

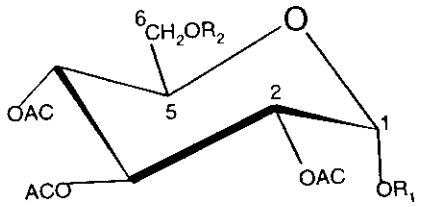

Figure 1. Molecular structures of reactant (GPA) and products.

1. GPA, $R_1 = R_2 =$ Ac.
2. SPKO-1, R_1 = stripped palm kernel mixed fatty acyl group; R_2 = Ac (mono-substituted product).
3. SPKO-2, $R_1 = R_2$ = stripped palm kernel mixed fatty acyl group (di-substituted product).
4. PO-1, R_1 = mixed palm fatty acyl group; R_2 = Ac (mono-substituted product).
5. C_{10}-2, $R_1 = R_2$ = capric acyl group (di-substituted product).

of the ring protons and ring carbons of GPA, after interesterification, are given in *Table 1.* It is observed that the interesterification effect on the chemical shifts of the ring protons, in all products, is minimal, ranging from +0.01 to -0.03 ppm. This minimal influence is expected, given that ring protons are 3-bond distance from the carbonyl carbon (substitution site). The changes are however random and do not reflect the positions of substitution in all the products. The changes in chemical shifts of ring carbons (y-carbon) however show much larger values, ranging from -0.12 to +0.21 ppm.

In all products, the anomeric carbon (C-l) experience upfield shifts. This reflects the substitution of fatty acyl group for the acetyl group. The fatty acyl group, which is electron richer, shields the anomeric carbon electromagnetically, leading to upfield shift (Schneider and Hoppen, 1978). The presence of the fatty acyl group, however, causes downfield shifts in all the other ring carbons *(Table 1).* This can only be explained in terms of a through-space non-bonded steric influence of the fatty acyl group. In SPKO-2 and C_{10}-2, where a second fatty acyl is attached to C-6, changes in chemical shifts do not however reflect this second substitution. *This* therefore constitute the limitation to the use of SIC on the pyranosyl ring proton and ring carbon atoms for structural elucidation. The

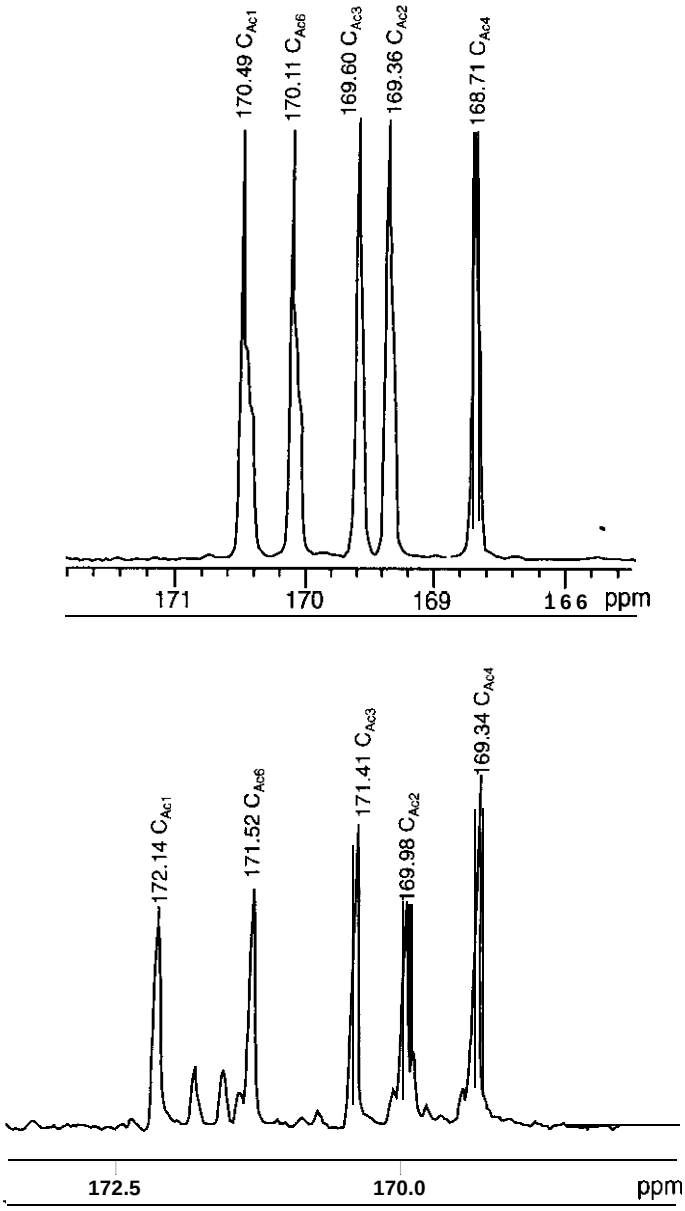

Figure 2. The ^{13}C-chemical shift of the carbonyl carbon of GPA (a), and SPKO-2 (b), showing relative changes in the δ_c values after fatty acyl substitution.

information obtained are thus not useful for assigning the positions of substitution.

. Interesterification effects on T-chemical shifts of the carbonyl carbons are give in *Table* 2. The α-deshielding (+ve difference) observed on the anomeric carbonyl carbons (C_{Ac1}) of all products are clearly distinguishable. The observed SIC changes are not only significant but consistent with respect to the positions of fatty acyl substituents. For instance, $\Delta\delta C_{Ac1}$ for all the

TABLE 1. RING CARBON AND PROTON CHEMICAL SHIFT DIFFERENCES (in ppm), ON INTERESTERIFICATION, RELATIVE TO GPA ($\Delta\delta_i = \delta_{i\ product} - \delta_{GPA}$)

		GPA	SPKO-1(Δδ)	SPKO-2(Δδ)	PO-1(Δδ)	C_{10}-2(Δδ)
Ring protons	H-1	6.33	6.33 (0.0)	6.34(+0.01)	6.34(+0.01)	6.33 (0.0)
	H-2	5.11	5.12 (+0.01)	5.10 (-0.01)	5.10 (-0.01)	5.12 (+0.01)
	H-3	5.47	5.45 (-0.021	5.47 (0.0)	5.46 (-0.01)	5.45 (-0.02)
	H-4	5.15	5.14 (-0.01)	5.12 (-0.03)	5.12 (-0.03)	5.14 (-0.01)
	H-5	4.11	4.12 (+0.01)	4.10 (-0.01)	4.11 (0.0)	4.12 (+0.01)
	H-6a	4.27	4.28 (+0.01)	4.28(+0.01)	4.28(+0.01)	4.28 (+0.01)
	H-6b	4.08	4.08 (0.0)	4.08 (0.0)	4.09(+0.01)	4.08 (0.0)
Ring carbons	C-1	89.06	88.94 (-0.12)	88.95 (-0.11)	88.96 (-0.10)	88.98 (-0.08)
	c-2	69.23	69.40 (+0.17)	69.44(+0.21)	69.44(+0.21)	69.43 (+0.20)
	c-3	69.86	70.01 (+0.15)	70.01(+0.15)	70.03(+0.17)	70.05 (+0.19)
	c-4	67.95	68.06 (+0.11)	68.08(+0.13)	68.08(+0.13)	68.10 (+0.15)
	c-5	69.86	70.01 (+0.15)	70.01(+0.15)	70.03(+0.17)	70.10 (+0.19)
	C-6	61.51	61.65 (+0.14)	61.65(+0.14)	61.65(+0.14)	61.70 (+0.19)
Proton No. observed[b]		22.52	46.10	75.46	53.61	54.38
Actual No. of protons[c]		22.00	46.70	74.40	53.53	54.00

[a]Ring protons in GPA were assigned according to those previously made by Utamura et al. (1966).
[b]From normalized ^1H NMR integration data.
[c]The number of protons based on the assumed molecular structure.

compounds studied are in the range 0.90 to 1.65 and are the highest in each product. This reflects the substitution of the fatty acyl group for the acetyl group at C-l position. Similarly, for SPKO-2 and C_{10}-2, A.6 for C_{Ac6} = 1.41 and 1.42, respectively. These are attributed to the second fatty acyl substituent at C-6. The SIC on C_{Ac1} and C_{Ac6}, in contrast to C-l and C-6, clearly distinguish between the mono- and the di-fatty acyl substitution products. This result is in good agreement with those reported (Bols, 1996; Obaje et al., 1999), and supported by the ^1H integration data in ***Table 1.***

Besides circumventing the problem of intractable changes in ^1H and W-chemical shift patterns in polyacetylated pyranoses (Utamura et *al.*, 1986), this method is simple, requiring only the ^{13}C NMR spectra of the reactant and product esters. In combination with structural information obtainable from the usual ^1H and ^{13}C NMR spectra, SIC on carbonyl carbons may allow for a comprehensive structural elucidation

TABLE 2. CARBONYL CARBON CHEMICAL SHIFT DIFFERENCES (in ppm) ON INTERESTERIFICATION, RELATIVE TO GPA ($\Delta\delta_i = \delta_{i\ product} - \delta_{GPA}$)

Carbonyl carbon	GPA	SPKO-1 (Δδ)	SPKO-2(Δδ)	PO-1(Δδ)	C_{10}-2(Δδ)
C_{Ac1}	170.49	171.39(+0.90)	172.14(+1.65)	171.42(+0.93)	172.14(+1.65)
C_{Ac2}	169.36	169.44(+0.08)	169.98(+0.62)	169.46(+0.10)	169.45(+0.09)
C_{Ac3}	169.60	169.95(+0.35)	170.41(+0.81)	170.04(+0.44)	170.34(+0.74)
C_{Ac4}	168.71	169.29(+0.58)	169.34(+0.63)	169.04(+0.33)	169.34(+0.63)
C_{Ac6}	170.11	170.31(+0.20)	171.52(+1.41)	170.39(+0.28)	171.53(+1.42)

of fatty acid esters of polyacetylated mono- and oligosaccharides using NMR spectroscopy.

REFERENCES

AKOH, C C and SWANSON, B G (1987). One-stage synthesis ofraffinose fatty acid polyesters. J. Food Sci., *52:*1570-1576.

BOLS, M (1996). ***Carbohydrate Building Blocks.*** John Wiley & Sons, Inc. New York. 29pp.

KHAN, R; KONOWICZ, P A; GARDOSSI, L; MATULOVA, M and de GENNARO, S (1996). Regioselective deacetylation of fully acetylated mono- and disaccharides with hydrazine hydrate. ***Australian J. Chem.,*** *49:*293-298.

KING-MORRIS, M J and SERIANNI, A S (1987). ^{13}C NMR studies of [1-^{13}C] aldoses: empirical rules correlating pyranose ring configuration and conformation with ^{13}C chemical shifts and ^{13}C-^{13}C spin couplings. ***J. Am. Oil Chem. Soc.,*** *109:*3501-3507.

NAKAMURA, S (1997). Using sucrose esters as food emulsifiers. ***INFORM,*** *8(8):*866-874.

OBAJE, 0 J; DZULKEFLY, K; ANUAR, K, ZAIZI, D and HAMDAN, S (1999). Solvent-free interesterification process for the synthesis of acetylated glucose fatty esters. ***Sci. Znt. (Lahore),*** *11 (1):*1-4.

SCHNEIDER, H J and HOPPEN, V (1978). Carbon-13 nuclear magnetic resonance substituent-induced shielding and conformational equilibria in cyclohexanes. ***J. Org. Chem.,*** *43:*3866-3873 (1978).

TERUI, Y; TORI, K and TSUJI, N (1976). Esterification shifts in C-13 NMR spectra of alcohols. ***Tetrahedron Letters,*** *8:*621-622.

UTAMURA, T; KUROMATSU, K; SUWA, K KOIZUMI, K and SHINGU, T (1986). Synthesis and ^{1}H- and ^{13}C-nuclear magnetic resonance spectra of all positional isomers of tetra-O-acetyl-D-glucopyranoses, and their monobenzyl and monotrityl derivatives. ***Chem. Pharm. Bull.,*** *34:*2341-2353.