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SYNTHESIS AND CHARACTERIZATION OF ACETYLATED GLUCOSE' FATTY ESTERS FROM PALM AND PALM KERNEL OIL FATTY METHYL ESTERS

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Acetylated glucose fatty esters (AGFE) were synthesized from palm and palm kernel fatty acid methyl esters (FAME) through a one-stage solvent-free interesterification reaction with glucose pentaacetate (GPA). Two main products obtained and identified were mono- and di-substituted AGFE. The hydrophile-lipophile balance (HLB), ternary phase diagram, cytotoxicity and antimicrobial bioassays were studied.

INTRODUCTION

Sugar-based esters of fatty acids are nonionic surfactants which have attracted attention from cosmetics, pharmaceutical, surfactant and nutrition scientists because of their multi-functional properties and safety. They are nontoxic, odourless, tasteless and biodegradable. The potential applications of sugar-based esters have however been greatly hindered by the use of toxic solvents, such as pyridine, dimethyl formamide, and dimethyl sulphoxide, during synthesis and processing (Weiss *et al.*, 1972). The numerous methods developed, so far, for the acylation of these polyhydroxylic systems are still faced with three fundamental problems: (i) finding a nontoxic mutual solvent for both the fatty acids and sugar, (ii) avoiding caramelization of the sugar at reaction temperatures and (iii) the cost of processing. In this report, we present results of a solvent-free, low temperature interesterification method. The HLB, phase diagram and biological assays of the interesterification reaction products are also presented.

MATERIALS AND METHODS

Materials

GPA (99% pure) was purchased from Fluka Biochemika, Buchs, Switzerland. Silica gel 60 (particle size 0.063-0.200 mm; 70-230 mesh ASTM) and pre-coated silica gel 60 F_{254} TLC plates were obtained from E. Merck (Darmstadt,

Germany). FAME of palm (PO) and palm kernel (PKO) fatty acids were gifts from Henkel Rika Oleochemicals, Malaysia. All solvents used were of analytical grade and obtained from E. Merck (Darmstadt, Germany).

Synthesis

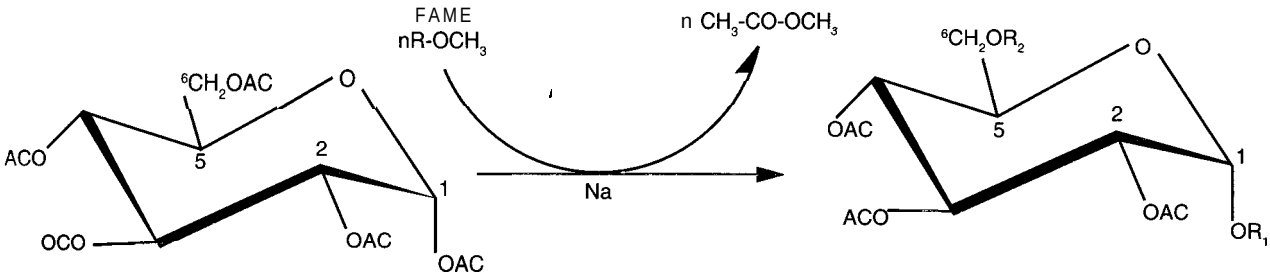
GPA was reacted with the appropriate FAME of PO or PKO, according to the method described by Akoh and Swanson (1987), with modifications. The interesteritication reaction is presented in ***Figure*** 1. Typically, FAME (15.0 mmol) was added into a three-necked, round-bottom flask equipped with a magnetic stirrer, stopcocks, 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 catalyst (0.12 g) were added and heating commenced with continuous stirring. An oil bath was used to maintain the temperature between 80°C and 100°C. The reaction was continued for 4 hr to 6 hr. The product was neutralized with 1-3 ml glacial acetic acid, allowed to cool, dissolved in acetonitrile and decolourized with 2 g of activated charcoal.

Preliminary investigations on TLC and HPLC indicated the presence of two products which were then separated in a slurry packed column (40 cm length x 2 cm i.d. and packed with silica gel 60, particle size 0.063-0.200 mm; 70-230 mesh ASTM). The first product, mono-fatty acyl glucose tetraacetate, was eluted with 20% ethanol in hexane (v/v), while the second product, di-fatty acyl triacetate, was eluted with 10% ethyl acetate in hexane (v/v). The solvents were evaporated by rotary evaporation to give a- and β-anomers of the products.

Molecular structures of products were established using FT-IR and, ^1H and ^{13}C-NMR spectrometric methods. ^1H and ^{13}C-NMR were run, on a Bruker DRX 300 spectrometer using tetramethylsilane (TMS) as internal standard. FT-IR spectra were obtained on a Perkin Elmer FT-IR 1725X spectrometer (Norwalk, Connecticut, USA). The melting points (m.p) were determined using a differential scanning calorimeter (DSC), model DSC-7, supplied by Perkin-Elmer (Norwalk, CT). Viscosity was measured using a cannon-ubbelohde semi-dilution viscometer, and densities were determined with a laboratory picnometer.

HLB

HLB was determined by the water number method (Gupta *et* al., 1983). Known surfactant samples covering the HLB range 1.8–16.0 were used to obtain a calibration graph. Solutions of samples, containing 1 g sample in 25 ml mixture of dimethylformamide (DMF) and benzene (95:5 v/v>, were titrated with distilled water



GPA, 1 *2-4*

Notes:
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.

Figure 1. Reaction scheme for the interesterification reaction ***of*** *GPA and FAME products.*

until permanent turbidity. HLB values of AGFE were obtained by interpolation on the calibration curve.

Ternary Phase Diagrams

Ternary phase diagrams of water/AGFE/ alcohols system were constructed by titrating various AGFE-alcohol mixtures with double distilled water. Thorough mixing was ensured by vortexing the mixture on a Thermolyne 37600 mixer. The micellar (isotropic regions) were determined by visual inspections for the change from turbid to transparent solution.

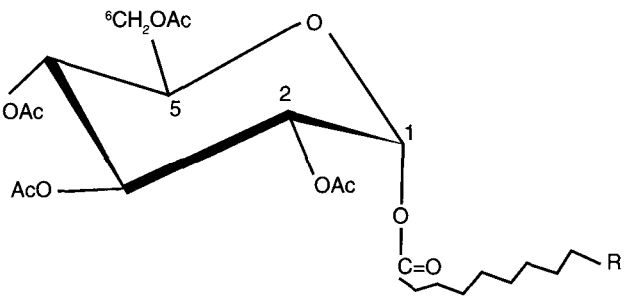
Cytotoxic and Antimicrobial Assays

The cytotoxic effect of the products (PKO-1, PKO-2 and PO-l) were evaluated on different cancer and normal cell lines. The HT-29 cell line (colon carcinoma) and 3T3 cell line (normal mouse fibroblast) were obtained from RIKEN Cell Bank, Japan. The CEM-SS cell line (T-cell lymphoblastic leukemia) was obtained from the National Cancer Institute, Maryland, USA. Cell viability was determined by microtitration assay (Ali *et al.*, 1997; 1998). Cytotoxicity was recorded as 50% inhibition concentration (IC_{50}) with reference to the untreated control.

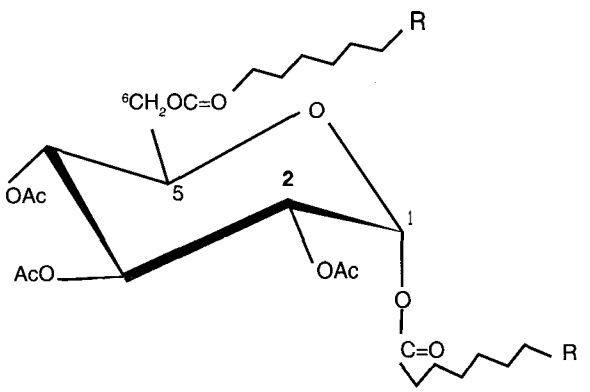
The antimicrobial activity was assayed using four microbial strains, *i.e. Pseudomonas aeruginosa* (Gram-negative), methycillin resistant *Staphylococcus aureus* (MRSA), and *Bacillus subtilis* B28 and B29, obtained from the culture collection of Applied Microbiology Laboratory, Faculty of Agriculture, Hokkaido University, Sapporo, Japan. The fungi collections were *Sacchromyces lipolytica* (yeast), *Candida albicans*, *Candida lipolytica* 2075 obtained from Department of Medical Microbiology and Immunology, Faculty of Medicine, UKM, Malaysia. Antimicrobial activity was determined by the disc diffusion method (Bauer *et al.*, 1966; Wilkins *et al.*, 1972). The growth-inhibition zone around the disk and the diameter of the inhibition zone was measured by a vernier caliper.

RESULTS AND DISCUSSION

Products, which were separated by column chromatography, were characterized by FT-IR, ^{1}H and ^{13}C-NMR spectrometry, and ^{1}H integration data. 2D H,C-COSY NMR was used to assign glucose-ring carbon atoms and heteronuclear multiple bond correlation (HMBC) was used to assign the carbonyl carbon atoms. The number and position of the fatty acyl substituents were determined by changes in ^{13}C-chemical shift of the carbonyl carbon atoms after the reaction. The chemical shift changes clearly showed the fatty acyl group to be on C_1 for the mono- and on C, and C_6 for the di-substituted products. From the analysis, the molecular structures of the mono-substituted AGFE was represented by molecular structure A *(Figure 2)*, while the di-substituted AGFE was represented by molecular structure B *(Figure 2)*.



Product A: 1 -O-fatty acyl 2,3,4,6-acetyl a-D-glucopyranose (~~R = the fatty acyl group).



Product B: 1, 6-O-fatty acyl 2,3,4-acetyl a-D-glucopyranose (~~R = the fatty acyl group).

Figure 2. Molecular structures ***of*** *AGFE products.*

The important features of the ^{1}H and ^{13}C-NMR are given bellow:

Mono substituted product: l-O-fatty acyl-1,2,3,4-tetraacetyl-α-D- glucopyranose

^{1}H NMR(400MHz, $CDCl_3$, 25°C, TMS): δ(ppm)=0.18[m,3H;$C\underline{H}_3$]; 0.40[m,18H; $(C\underline{H}_2)_4$,$(C\underline{H}_2)_6$]; 0.96[m,2H; $C\underline{H}_2$-CH_2-CO];

1.2-1.4[m,12H; $C\underline{H}_3$-CO]; 1.71[t, 2H;$C\underline{H}_2$-CO]; 3.35[1H,H_{6b}];3.4[m, 1H,H5]; 3.56[m,1H;H_{6a}], 4.38[1H,H_2]; 4.41[t,1H;H_4,]; 4.74[t,1H; H_3]; 6.33 and 5.61[d,1H,$H_1\alpha$ and $H_{1\beta}$ respectively] 5.09[m,2H;$C\underline{H}$=$C\underline{H}$]; and 7.3[$CDCl_3$] as solvent.

^{13}C NMR (400MHz, $CDCl_3$, 25°C, TMS): δ(ppm)=13.66[$\underline{C}H_3$-CH_2-]; 19.72-20.29,[$\underline{C}H_3$-CO]; 28.43-29.35[(-$\underline{C}H_2$-)$_n$]; 31.41[$\underline{C}H_2$-CO] 61.08[1°$\underline{C}_6$ of glucose]; 67.52[C_4]; 68.86[C_2] 69.90[C_3 and C,]; 88.39[C_1]; 168.86-170.71[$\underline{C}$=O]; 129.76,130.02[$\underline{C}$=$\underline{C}$]; 75.2-78.8[$\underline{C}DCl_3$].

Di-substituted product: 1,6-O-di-fatty acyl-2,3,4-triacetyl-a-D-glucopyranose

^1H NMR(400MHz, $CDCl_3$, 25°C, TMS): δ(ppm)=0.18[m,6H;$C\underline{H}_3$]; 0.40[m,40H; $(C\underline{H}_2)_4$, $(CH_2)_6$]; 0.96[m,4H; $C\underline{H}_2$-CH_2-CO]; 1.2-1.4[m,9H; $C\underline{H}_3$-CO]; 1.4[m,8H; $C\underline{H}_2$-CH=CH-$C\underline{H}_2$]; 1.71[t, 4H;$C\underline{H}_2$-CO]; 3.35[1H,H_{6b}]; 3.4[m, 1H,H_5]; 3.56[m,1H;H_{6a}], 4.38[1H,H_2]; 4.41[t,1H;H_4,]; 4.74[t,1H; H_3]; 6.33 and 5.61 [d,1H,$H_1\alpha$ and $H_{1\beta}$ respectively]; 5.09[m,4H; $C\underline{H}$=$C\underline{H}$]; and 7.3[$CDCl_3$] as solvent.

^{13}C NMR (300MHz, $CDCl_3$, 25°C, TMS): δ(ppm)=14.05[$\underline{C}H_3$-CH_2-]; 20.42, 20.54,20.64, 20.67,20.84[$\underline{C}H_3$-CO]; 22.69-34.1[(-$\underline{C}H_2$-)$_\eta$]; 61.55[1° $\underline{C}_6$ of glucose]; 67.95-69.84.77 [C_5,C_4,C_2 and C_3]; 89.11[C_1]; 168.91-170.98[$\underline{C}$=O]; 129.76,130.02[$\underline{C}$=$\underline{C}$]; 75.2-78.8[$\underline{C}DCl_3$].

The result showed that PKO methyl ester yielded 60.5% 1-0-(mono-substituted) acetylated glucose fatty ester (PKO-1) and 20.2% 1,6-O-(di-substituted) acetylated glucose fatty ester, (PKO-2), while PO methyl ester yielded mainly 90.2% 1-0-(mono-substituted) acetylated glucose fatty ester, (PO-l). A summary of the interesterification reaction conditions, yields, viscosities, melting points and anomeric compositions of the products is given in *Table* 1. The interesterification products obtained from PKO (i.e. PKO-1 and PKO-2) were light brown liquids while PO-l, obtained from PO, was a brown paste. No melting endotherm was observed for PKO-1 within the DSC thermogram range studied, but PKO-2 showed a melting endotherm at –6.5°C. PO-l showed a much higher melting point at 33.6°C.

All the products showed intermediate HLB values, ranging from 8.1 to 11.9 *(Table 2).* These HLB values indicate that the products are generally suitable as emulsifiers. While PKO-1 and PO-l with HLB values of 10.3 and 11.9, respectively, were most suitable as oil-in-water (O/W> solubilizers, PKO-2 with HLB of 8.1 was a suitable water-in-oil emulsifier.

Figure 3 shows the solubility/isotropic regions for the water/PKO-1/alcohol system. In the isotropic regions, the three components (water, PKO-1 and alcohol) were associated to form micellar aggregates. At such a point, the three component mixture formed a single (isotropic) phase and the solution became transparent. Propan-1-ol formed the largest isotropic region, with the bulk of the isotropic region being along the 40:60 AGFE:alcohol line. In all cases, the isotropic regions protruded towards the alcohol apex. This information is useful in constructing pseudo ternary phase diagrams

TABLE 1. REACTION CONDITIONS[a] AND PRODUCT CHARACTERISTICS

Compound	Max. temp. (°C)	Yield[b] (%)	Viscosity[c]	m.p. (°C)	α-Anomer[d] (%)	β-Anomer[d] (%)
GPA			0.011	113.8	100	0
PKO-1	91	60.5	0.027	o.r	80.3	19.7
PKO-2	91	20.2	0.043	-6.5	71.4	28.6
PO-1	90	90.2	0.032	33.6	90.7	9.3

Notes:

[a] Mole ratio: GPA(1): FAME(3); reaction time, 6 hr and 0.5% (by weight) of Na-metal catalyst.

[b] Percentage weight of product per theoretical weight based on the initial weight of GPA.

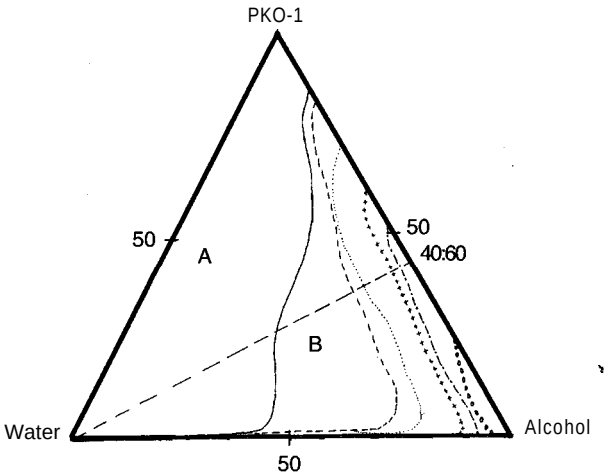
[c] Specific viscosity, η_{sp}, at 28°C.

[d] Calculated from the relative intensities of the corresponding anomeric proton signals in the ^1H n.m.r. spectra (δHα=6.33ppm; (δHβ=5.78ppm).

TABLE 2. HLB RESULTS

Compound	HLB	Application
PKO-1	10.3	O/W emulsion, solubilizer
PKO-2	8.1	W/O emulsion agent
PO-1	11.9	O/W emulsion, solubilizer

which are important for locating regions suitable for certain product formulations. This preliminary result showed that propan-1-ol is the best solubilizer for AGFE.



Notes:

(-) propan-l-ol, (--- -) ethanol, (.....) butanol, (+++++) pentan-l-ol, (.-.-.-.) 1-hexanol and (••••••••••) 1-octanol.

A: anisotropic solution; B: isotropic solution.

Figure 3. Ternary phase diagram of water/PKO-1/ alcohol system.

Table 3 shows the diameter of the inhibition zones of PKO-1, PKO-2 and PO-l against the target microbes. PKO-1 was only weakly active against *P. aeruginosa* with an inhibition zone diameter of 9.0 mm. PKO-2 showed a moderate activity against *P. aeruginosa* with an inhibition zone diameter of 14.0 mm and was weakly active against S. *aureus, B. subtilis* B28 and B29. PO-1 was only weakly active against *P. aeruginosa* and *B. subtilis* B29. Although these activities were generally weak, they were good enough to augment and enhance preservative measures in most formulations. These compounds can thus perform dual roles as emulsifiers and preservatives.

Microscopic examination of all the treated cells during and after a three-day incubation showed no cell reduction. The treated cells remained identical with the reference cell at the end of the incubation time. The non-cytotoxicity of the three products towards cancer and normal cell lines showed that AGFE could be used in food applications.

TABLE 3. DIAMETER OF MICROBIAL INHIBITION ZONE (mm) OF AGFE AGAINST TARGET MICROBES

Microbe	PKO-1	PKO-2	PO-1
P.aeruginosa	9 mm	14 mm	7 mm
S.aureus (MRSA)	-	7 mm	-
B.subtilis B28	-	7 mm	-
B.subtilis B29	-	7 mm	7 mm

CONCLUSION

Solvent-free interesterification provided a convenient route to the production of food-grade glucose fatty esters. This methods produced nontoxic (solvent residue-free) products, thus expanding the scope for application of products. The low temperature requirement for this process reduces the risks of degradation and denaturing of reactants and reduced energy cost. The reaction products, mono- and di-fatty acid acetylated glucose esters, were potential emulsifiers. This was indicated by their HLB values. Biological assessments showed AGFE to be non cytotoxic to human and animal cells, rather, they were antimicrobial, albeit mildly.

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