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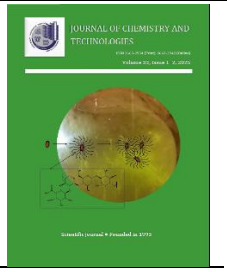

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PHYSICO-CHEMICAL STUDY, MOLECULAR MODELING AND ANTIBACTERIAL ACTIVITY OF α- AND β-ANOMERS OF XYLOSE ESTERS

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Abstract

Non-ionic surfactants obtained from renewable resources represent a new challenge in biotechnology and have a range of applications in many industries. In this context, the α- and β-anomers of synthesized D-xylose-based biosurfactants with various chain length were easily separated and characterized through analytical methods. Their surface and emulsifying properties were evaluated. To study the reactivity of the two anomers of synthetized biosurfactants, the spatial conformations of the prepared acyl-xylopyranose were obtained by molecular modeling with Gaussian 9 software using the density functional theory method. Compounds α are the softest so more reactive than the β ones. The antibacterial activity of sugar fatty acid ester anomers was also studied. The results obtained indicate the importance of anomeric form and chain length for the stability of the synthesized compounds.

Keywords: Xylose ester; anomers; surface properties; modeling; antibacterial activity.

ФІЗИКО-ХІМІЧНІ ДОСЛІДЖЕННЯ, МОЛЕКУЛЯРНЕ МОДЕЛЮВАННЯ ТА АНТИБАКТЕРІАЛЬНА АКТИВНІСТЬ α- ТА β-АНОМЕРІВ ЕСТЕРІВ КСИЛОЗИ

Лулу Фатма, Біджу-Хайур Чахра

Лабораторія органічного синтезу, моделювання та оптимізації хімічних процесів (LOMOP), природничий факультет, Університет Баджі-Мохтар, BP 12 El-Hadjar, 23000 Annaba, Алжир

Анотація

Отримання неіоногенних поверхнево-активних речовин з відновлюваних ресурсів є новим викликом у біотехнології, оскільки ці сполуки мають широкий спектр застосувань у багатьох галузях промисловості. У цьому контексті α- та β-аномери синтезованих біологічно активних речовин на основі D-ксилози з різною довжиною ланцюга були легко розділені та охарактеризовані за допомогою аналітичних методів. Оцінені їх поверхневі та емульгуючі властивості. Для вивчення реакційної здатності двох аномерів, синтезованих біосурфактантів, методом молекулярного моделювання за допомогою програми Gaussian 9 з використанням методу теорії функціоналу густини було отримано просторові конформації одержаної ацил-ксилопіранози. α-Сполуки є найбільш гнучкими, тому більш реакційноздатними, ніж β. Також досліджено антибактеріальну активність аномерів естерів цукрових жирних кислот. Одержані результати свідчать про важливість аномерної форми та довжини ланцюга для стабільності синтезованих сполук.

Ключові слова: естер ксилози; аномери; поверхневі властивості; моделювання; антибактеріальна активність.

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Introduction

Sugar Fatty Acid Esters (SFAEs) are of great interest in the field of biocompatible components [1]. These molecules constitute interesting nonionic bio-surfactants. They are made from renewable, inexpensive, and readily available raw materials [2]. Because of their amphiphilic nature, non-toxicity and bio-degradability, they are often used in the pharmaceutical, cosmetic and food industries [3; 4]. SFAE can be synthesized using either chemical or biological catalysts. As a result of regioselectivity and mild reaction conditions of enzymatic process [5], there is growing interest in the application of lipases as biocatalysts for carbohydrate fatty acid esters production [6; 7]. Numerous reports have shown that lipases are good biocatalyst in nonaqueous media [8].

In this study, we report the anomeric separation of α- and β-anomers of xylose esters incorporating C_{12} and C_{18} chains. Xylose laurate and xylose stearate were synthesized by the enzymatic way [9]. The anomerization process yielded esters of the monosaccharide. At the same time, the surfactant properties (including air-water surface tension, critical micellar concentration and emulsion power and stability) of the anomeric forms were determined. These compounds were obtained by acetylation of synthesized xylose esters and subsequent deacetylation of the resulting peracetylated anomeric forms [10; 11], a protocol that reliably generates the required esters in high yield and with excellent regioselectivity.

Molecular modeling was also applied using DFT/B3LYP method with 6-31G (d,p) basis set [12]. Stability of α- and β-anomers of 1-*O*-acylxylose esters derivatives has been analyzed to understand the forces involved in the stability of these compounds.

On the other hand, the antibacterial activities of α- and β-anomers of xylose esters against common food-related bacteria were determined by measuring the diameters of growth inhibition zones and minimum inhibitory concentration (MIC). Indeed, sugar fatty acid esters have attracted much attention in recent years because of their biological properties [13].

Material and Methods

Materials

Substrates (D-xylose, dodecanoic acid, octadecanoic acid), solvents and other chemicals were obtained from Sigma-Aldrich and Merck. *Candida antarctica* type B lipase immobilized on acrylic resin was purchased from Novo Nordisk (Bagsvaerd, Denmark). All reagents used in this study were analytical grade.

Methods

The ^{1}H and ^{13}C NMR spectrum were carried out using a Bruker A 400 apparatus in $CDCl_3$ and DMSO-d6 with a frequency of 400 MHz.

The infrared spectroscopic FTIR (ATR) analysis in the range from 4000 cm^{-1} to 370 cm^{-1} was carried out using a Bruker apparatus. All the spectra were recorded with the transmission mode.

Xylose esters synthesis

D-xylose (1g, 6.66 mmol) was first dissolved in 25 mL of ethylmethylketone (EMK) for one night, at 60°C. After that, the acyl donor was added: lauric acid (4.42 g, 13.32 mmol) or stearic acid (5.56g, 13.32 mmol). The biocatalyst (300 mg of CAL B) and molecular sieves 4A° (300 mg) were then incorporated. Aliquots were removed at intervals, filtered and analyzed quantitatively by volumetric titration. At the end of the reaction, the lipase was removed by filtration and the solvent was evaporated to dryness under reduced pressure. The enzymatic products were found to be a mixture of α- and β-anomers.

Acetylation of xylose esters

Synthesized esters from D-xylose were acetylated with acetic anhydride (12 equivalents) and sodium acetate (6 equivalents) for 2 hours at 50 °C under stirring. The reaction was followed by thin-layer chromatography (TLC). The crude reaction mass was transferred to diethyl ether (50 mL) and washed several times with saturated Na_2CO_3 solution until neutral pH was reached.The organic phase was then isolated, dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, eluting mixture petroleum ether/AcOEt 8.5:1.5).

Deacetylation of acetylated anomeric xylose esters

The peracetylated compound was dissolved in CH_2Cl_2/MeOH (1/1) mixture. Then sodium methylate (6 equivalents) was added to the medium and the release was monitored by TLC. At the end of the reaction an ion-exchange resin was added to neutralize the excess of sodium methylate. The resulting mixture was then filtered and the solvent evaporated under reduced pressure. The two anomers were then separated by flash chromatography.

Measurement of surface tension

The surface tension values (γ) were measured at 25 ˚C by the pendant drop method using an IT

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concept Tracker drop tensiometer. The critical micellar concentration (CMC) was calculated from the breaking point in γ versus $\log_{10}$ concentration plots. γ_{CMC} is the surface tension parameter corresponding to the CMC, the concentration of surfactant above which micelles form.

Emulsion power and stability

Aqueous monoester solutions (4 mL) of different concentrations were prepared. The samples were homogenized with 1ml of paraffin oil, at 8000 rpm with a shear mixer, for 15 min at 25 ˚C. The emulsifying ability (EA) and emulsion stability (ES) were calculated using Equation 1 and 2.

$$EA\ (\%) = \frac{H_1}{H_0} \text{x} 100 \qquad (1)$$

$$ES\ (\%) = \frac{H_2}{H_1} \text{x} 100 \qquad (2)$$

Where H_0 is the initial height of solutions and paraffin oil, H_1 is the height of the emulsion layer measured immediately after homogenization. H_2 is the height of the emulsion layer after 2, 24, 48 and 72 hours.

Molecular modeling

All the computations were carried out using GAUSSIAN 09 software. The DFT modeling method, using the hybrid B3LYP functional, was used to calculate theoretical parameters with the basis set combination 6-31 G (d, p) and 6-31 G (d). Geometry optimization was carried out until global minima were achieved.

Antibacterial Activity

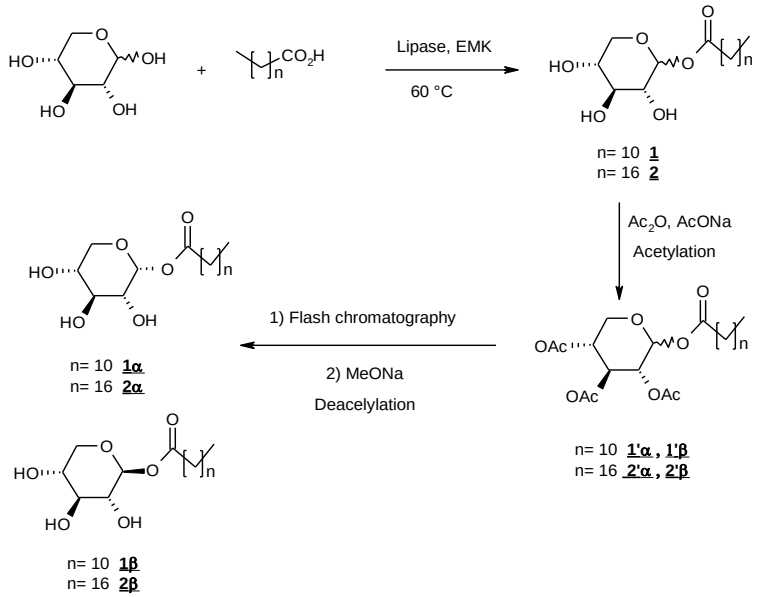
The antibacterial activity of sugar fatty acid esters was determined by the method described by Kirby-Bauer. Mueller Hinton agar (pH 7.2, 4mm depth) Petri dishes were inoculated with test organisms. Watman paper 6 mm diameter was impregnated with a volume of 20 µL of the tested molecules and deposited on the surface of the agar. Same volume of 20 µL dimethylsulfoxide was dispensed into the control well. Thus, each diffuse concentration in the agar forms an inhibition zone that will be measured 24 h after incubation at 37 °C by ruler caliper.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of micro-organism after incubation. To determinate the MIC of each compound a dilution range of fatty acid sugar esters were prepared as follows: (512, 256, 128, 64, 32, 16, 4, 2, 1, 0.5, 0.25 µg/mL). Then these concentrations are distributed to test tubes of nutrient broth seeded with a known concentration of the bacterial strain. We put the tubes in the oven at 37°C. Bacterial growth will be evaluated according to the OD obtained. The reading is done by comparison with the control tube; the dilution which gives the first clear tube (without bacterial growth), determines the MIC.

Results and discussion

Synthesis of α,β-D-xylopyranose esters **1** and **2** was performed with lauric (dodecanoic) and stearic (octadecanoic) acids by enzymatic esterification using a lipase. At 60°C under thermodynamic control, major pyranic forms were obtained. α- and β-anomers were obtained in pure forms by *O*-acetylation and then *O*-deacetylation of the corresponding acetylated α- and β-xylopyrannose **1'** and **2'** previously prepared (Scheme 1).



Scheme 1. Synthesis of 1-*O*-acylxylopyranose esters.

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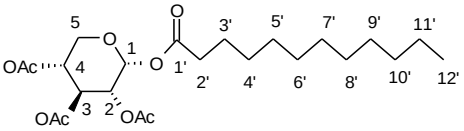
The products are finally purified and separated by flash chromatography and their chemical structures are confirmed using ^{1}H NMR, ^{13}C NMR and FTIR-ATR.

1-O-Dodecanoyl-2,3,4-tri-O-acetyl- α-D-xylopyranose (**1'α**)



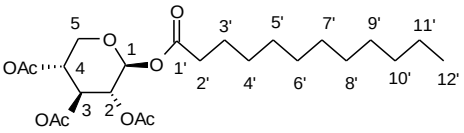
1-*O*-Dodecanoyl-2,3,4-tri-*O*-acetyl- α-D-xylopyranose (**1'α**) was obtained as a white powder following the general procedure previously described with 1-*O*-dodecanoyl-α,β-D-xylopyranose (1.974g of 0.006 mol) in 7.22 ml acetic anhydride (12 equiv) and sodium acetate (2.88g, 6 equiv). Reaction time: 3 hours. Yield: 49.53 %. RF: 0.35 (petroleum ether/AcOEt, 8.5:1.5 v/v).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 5.48 (t, J = 9.8 Hz, 1H), 5.08 – 4.93 (m, 2H), 4.79 (dd, J = 10.1, 3.3 Hz, 1H), 3.85 – 3.70 (m, 1H), 3.68 – 3.53 (m, 2H), 3.38 (d, J = 9.8, 6.6 Hz, 1H), 2.05 (s, 9H), 1.63 (t, 2H), 1.41 – 1.07 (m, 18H), 0.88 (t, J = 6.8 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$): 170.09 ($CH_3\underline{\mathbf{C}}O/\underline{\mathbf{C}}O$), 95.80($C_1$), 71.37($C_2$), 69.93($C_3$), 68.68($C_4$), 58.39($C_5$), 32.02($C_{2'}$), 29.73($C_{3'}$, $C_{4'}$, $C_{5'}$, $C_{6'}$, $C_{7'}$, $C_{8'}$, $C_{9'}$), 26.15($C_{10'}$), 22.78($C_{11'}$), 20.78(3$\underline{\mathbf{C}}H_3CO$), 14.18 ($C_{12'}$).

IR (ATR)**:** 2850.76, 2918.79, 1732.17, 1468.30, 1230.10, 1369.69.

1-O-Dodecanoyl-2,3,4-tri-O-acetyl-β-D-xylopyranose (**1'β**)



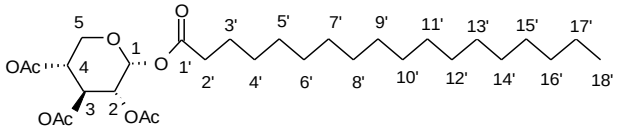
1-*O*-Dodecanoyl-2,3,4-tri-*O*-acetyl-β-D-xylopyranose **(1'β)** was obtained as a white powder following the general procedure previously described with 1-*O*-dodecanoyl-α,β-D-xylopyranose (1.974 g of 0.006 mol) in acetic anhydride (12 equiv) and sodium acetate (2.88 g, 6 equiv). Reaction time: 3 hours. Yield: 50.46 %, RF: 0.25 (petroleum ether /AcOEt, 8.5:1.5 v/v).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 5.16 (t, J = 8.6, 1.5 Hz, 1H), 5.00 – 4.82 (m, 2H), 4.47 (d, J = 6.8 Hz, 1H), 4.23 – 4.06 (m, 1H), 3.89 – 3.72 (m, 1H), 3.58 – 3.15 (m, 2H), 2.04 (s, 9H), 1.56 (t, J = 7.0 Hz, 2H), 1.36 – 1.14 (m, 18H), 0.88 (t, 3H).

^{13}C NMR (101 MHz, $CDCl_3$): 169.72 ($CH_3\underline{\mathbf{C}}O/\underline{\mathbf{C}}O$), 100.66 ($C_1$), 71.55 ($C_2$), 70.90 ($C_3$), 68.97 ($C_4$), 61.99 ($C_5$), 31.86 ($C_{2'}$), 29.60 ($C_{3'}$, $C_{4'}$, $C_{5'}$, $C_{6'}$, $C_{7'}$, $C_{8'}$, $C_{9'}$), 25.86 ($C_{10'}$), 22.62 ($C_{11'}$), 20.62 (3$\underline{\mathbf{C}}H_3CO$), 14.03 ($C_{12'}$).

IR (ATR): 2849.12, 2916.05, 1752.36, 1466.07, 1214.94, 1370.27.

1-*O-Octadecanoyl-2,3,4-tri-O-acetyl-α-D-xylopyranose* (**2'α**)



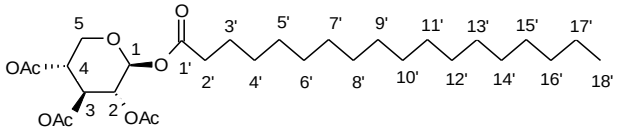
1-*O*-Octadecanoyl-2,3,4-tri-*O*-acetyl-α-D-xylopyranose (**2'α)** was obtained as a white powder following the general procedure previously described with 1-*O*-dodecanoyl-α,β-D-xylopyranose (1.7 g, 0.004 mol) in 5 ml acetic anhydride (12 equiv) and sodium acetate (2.88 g, 6 equiv). Reaction time: 3 hours. Yield: 53.40 %. RF: 0.35 (petroleum ether /AcOEt, 8.5:1.5 v/v).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 5.48 (t, J = 9.8 Hz, 1H), 5.08 – 4.93 (m, 2H), 4.79 (dd, J = 10.1, 3.3 Hz, 1H), 3.85 – 3.70 (m, 1H), 3.68 – 3.53 (m, 2H), 3.38 (d, J = 9.8, 6.6 Hz, 1H), 2.05 (s, 9H), 1.63 (t, 2H), 1.41 – 1.07 (m, 18H), 0.88 (t, J = 6.8 Hz, 3H.

^{13}C NMR (101 MHz, $CDCl_3$): 170.09 ($CH_3\underline{\mathbf{C}}O/\underline{\mathbf{C}}O$), 95.80 ($C_1$), 71.37 ($C_2$), 69.93 ($C_3$), 68.68 ($C_4$), 58.39 ($C_5$), 32.02 ($C_{2'}$), 29.73 ($C_{3'}$, $C_{4'}$, $C_{5'}$, $C_{6'}$, $C_{7'}$, $C_{8'}$, $C_{9'}$), 26.15 ($C_{10'}$), 22.78 ($C_{11'}$), 20.78 (3 $\underline{\mathbf{C}}H_3CO$), 14.18 ($C_{12'}$).

IR (ATR)**:** 2850.76, 2918.79, 1732.17, 1468.30, 1230.10, 1369.69.

1-*O-Octadecanoyl-2,3,4-tri-O-acetyl-β-D-xylopyranose* (**2'β**)



1-*O*-Octadecanoyl-2,3,4-tri-*O*-acetyl-β-D-xylopyranose (**2'β**) was obtained as a white powder following the general procedure previously described with 1-*O*-octadecanoyl-α,β-D-xylopyranose (1.7 g, 0.004 mol) in 5 ml acetic anhydride (12 equiv) and sodium acetate (1.96 g, 6 equiv). Reaction time: 3 hours. Yield: 67.29 %. RF: 0.30 (petroleum ether /AcOEt, 8.5:1.5 v/v).

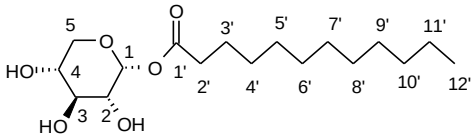
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 5.09 (t, J = 8.6 Hz, 1H), 4.97 – 4.78 (m, 2H), 4.40 (d, J = 6.8 Hz, 1H), 4.04 (dd, J = 11.8, 5.1 Hz, 1H), 3.73 (d, J = 9.7, 6.5 Hz, 1H), 3.45 – 3.19 (m, 2H), 1.97 (s, 9H), 1.49 (t, J = 7.1 Hz, 2H), 1.35 – 1.09 (m, 30H), 0.81 (t, J = 6.7 Hz, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$) :170.01 ($CH_3\underline{\mathbf{C}}O/\underline{\mathbf{C}}O$), 100.67 ($C_1$), 71.56 ($C_2$), 69.63 ($C_3$), 68.98 ($C_4$), 62.00 ($C_5$), 31.89 ($C_{2'}$), 29.66

($C_{3'}$,$C_{4'}$,$C_{5'}$,$C_{6'}$,$C_{7'}$,$C_{8'}$,$C_{9'}$,$C_{10'}$,$C_{11'}$,$C_{12'}$,$C_{13'}$,$C_{14'}$,$C_{15'}$), 25.87 ($C_{16'}$), 22.64 ($C_{17'}$), 20.66 (3**C**H_3CO), 14.04 ($C_{18'}$).

IR (ATR)**:** 2915.57, 2848.23, 1752.50, 1216.94, 1309.63.

1-*O-Dodecanoyl-α-D-xylopyranose* (**1α**)



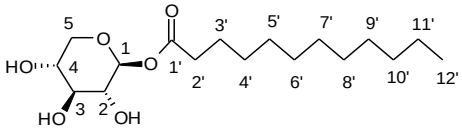
1-*O*-Dodecanoyl-α-D-xylopyranose (**1α**) was obtained as a white powder following the general procedure previously described with 1-*O*-dodecanoyl-2,3,4-tri-*O*-acetyl- α-D-xylopyranose (0.656g, 0.0014mol) in sodium methoxide (0.46 g, 0.008 mol). Yield: 88.84 %. Rf: 0.55 (CH_2Cl_2/MeOH, 9:1 v/v).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 4.69 (d, J = 3.5 Hz, 1H), 3.61 – 3.58 (m, 1H), 3.58 – 3.55 (m, 1H), 3.54 – 3.50 (m, 1H), 3.42 (d, J = 3.8 Hz, 1H), 3.40 (d, J = 4.1 Hz, 1H), 3.38 – 3.30 (m, 1H), 1.99 – 1.82 (m, 0H), 1.63 – 1.46 (m, 2H), 1.29 – 1.09 (m, 17H), 0.81 (t, 3H).

^{13}C NMR (101 MHz, CDCl3): 179.61(**C**O), 97.53 ($C_{1'}$), 71.17 ($C_{2'}$), 69.00 ($C_{3'}$), 67.49 ($C_{4'}$), 60.89 ($C_{5'}$), 30.93 (C_2), 28.70 (C_4, C_5, C_6, C_7, C_8, C_9), 25.08 (C_{10}), 21.68 (C_{11}), 13.07 (C_{12}).

IR (ATR): 3502.80, 2915.25, 2849.78, 1751.61, 1037.29.

1-*O-Dodecanoyl-β-D-xylopyranose* (**1β**)



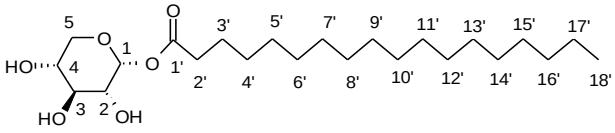
1-*O*-Dodecanoyl-β-D-xylopyranose (**1β**) was obtained as a white powder following the general procedure previously described with 1-*O*-dodecanoyl-2,3,4-tri-*O*-acetyl-β-D-xylopyranose (0.479g, 0.001mol) in sodium methoxide (0.32g, 0.006 mol). Yield: 92.48%. Rf: 0.35 (CH_2Cl_2/MeOH, 9:1 v/v).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 4.83 (s, 2H), 4.29 – 4.22 (m, 1H), 3.94 (dd, J = 11.6, 4.9 Hz, 1H), 3.87 – 3.76 (m, 1H), 3.70 – 3.59 (m, 1H), 3.51 (dd, J = 11.3, 5.3 Hz, 1H), 3.49 – 3.43 (m, 1H), 3.35 (t, J = 7.8 Hz, 1H), 3.24 (t, J = 10.7 Hz, 1H), 2.13 – 1.72 (m, 2H), 1.74 – 1.48 (m, 2H), 1.41 – 1.14 (m, 16H), 0.88 (t, J = 6.6 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6): 175.87(**C**O), 104.11 ($C_{1'}$), 77.14 ($C_{2'}$), 73.70 ($C_{3'}$), 68.99 ($4_{1'}$), 66.14 ($C_{5'}$), 31.76 (C_2), 29.48 (C_4, C_5, C_6, C_7, C_8, C_9), 25.97 (C_{10}), 22.54 (C_{11}), 14.35 (C_{12}).

IR (ATR)**:** 1639.35,1044.40, 3332.01, 2848.80, 2954.75.

1-O-Octadecanoyl-α-D-xylopyranose (**2α**)



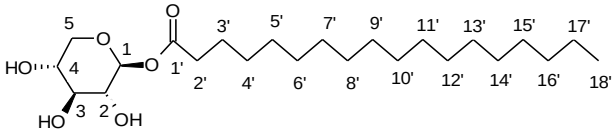
1-*O*-Octadecanoyl-α-D-xylopyranose (**2α**) was obtained as a white powder following the general procedure previously described with 1-*O*-octadecanoyl-2,3,4-tri-*O*-acetyl-α-D-xylopyranose (0.255 g, 0.00047 mol) in sodium methoxide (0.15g, 0.00282 mol). Yield: 60 %. Rf: 0.65 (CH_2Cl_2/MeOH, 9:1 v/v).

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 4.55 (d, J = 3.6 Hz, 1H), 3.53 (dt, J = 9.7, 6.7 Hz, 1H), 3.37 (q, J = 3.9 Hz, 1H), 3.34 – 3.32 (m, 1H), 3.31 – 3.28 (m, 6H), 3.26 (t, J = 4.8 Hz, 2H), 3.16 (dd, J = 9.3, 3.6 Hz, 1H), 1.66 – 1.55 (m, 1H), 1.54 – 1.46 (m, 2H), 1.39 – 1.10 (m, 26H), 0.85 (t, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) :179.62 (CH_3**C**O), 97.47 ($C_{1'}$), 71.19 ($C_{2'}$), 68.97 ($C_{3'}$), 67.46 ($C_{4'}$), 60.93 ($C_{5'}$), 30.93 (C_2), 28.72 (C_4, C_5,C_6,C_7,C_8,C_9,C_{10},C_{11},C_{12},C_{13},C_{14},C_{15},C_{16}), 25.09 (C_3), 21.68 (C_{17}), 13.07 (C_{18}).

IR (ATR)**:** 3501.31, 2914.01, 2849.61, 1642.93, 1038.57.

1-*O-Octadecanoyl-β-D-xylopyranose* (**2 β**)



1-*O*-Octadecanoyl-β-D-xylopyranose(**2 β**) was obtained as a white powder following the general procedure previously described with 1-*O*-octadecanoyl-2,3,4-tri-*O*-acetyl-β-D-xylopyranose (0.689 g, 0.0012 mol) in sodium methoxide (0.38 g, 0.0072 mol). Yield: 76.31 %. Rf: 0.57 (CH_2Cl_2/MeOH, 9:1 v/v).

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 3.93 – 2.62 (m, 10H), 2.26 (p, *J* = 1.9 Hz, 2H), 1.39 (s, 1H), 0.99 (s, 16H), 0.77 – 0.48 (m, 3H).

^{13}C NMR (101MHz, DMSO-d_6): 174.76 (**C**O), 103.87 ($C_{1'}$), 76.92 ($C_{2'}$), 73.45 ($C_{3'}$), 68.69 ($C_{4'}$), 65.90 ($C_{5'}$), 31.46 (C_2), 29.19 (C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}), 25.70 (C_3), 22.25 (C_{17}), 14.09 (C_{18}).

IR (ATR): 3377.41, 2914.27, 2848.37, 1561.21,1040.40.

Surface active properties

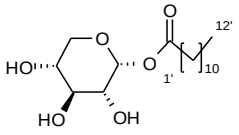
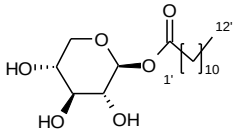
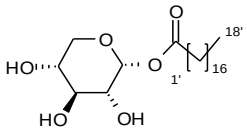
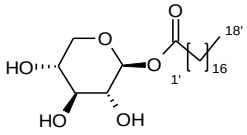
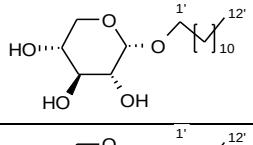
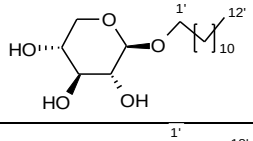
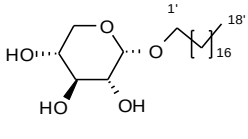
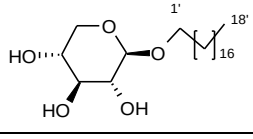
Surface tension (*γ*) and critical micellar concentration (CMC) of synthesized compounds **1α**, **1β, 2α** and **2β** have been determined. The

surface active properties of compounds **1** and **2** have already been reported in the literature [9] and have shown their potential as biosurfactant since they have ability to reduce the surface tension.

In this study we demonstrated that acyl-α-D-xylopyranoses esters (Table 1, entries 1–4) have the best surface properties and exhibited a greater surface activity than the corresponding β-anomers at high CMC due to the high solubility of these surfactants in water (0.16 mmol/L for **1α** and 0.10 for **2α**). The results for the obtained alkylxylosides (Table 1, entries 5–8) in a previous study [14] showed that alkyl β-D-xylosides have the best surface properties. On the contrary, in the course of this study and for the synthesized acylxyloses esters, the obtained results for the surface properties showed that the α-anomers are more active than the β-anomers. This can be explained by the increased hydrophilicity of the synthesized compounds by the addition of the carbonyl group. Introducing a cabonyl group in the alkyl chain change radically the surface activity of the xylose based-biosurfactants with the same lenght of the chain [15]. The obtained values of CMC and γ (Table 1, entries 1–4) are in accordance with the reported results concerning the acylxyloses esters [16]. The results showed that the carbon chain was the most important factor influencing the surface properties.

Table 1

Surface tension characteristics of acylxylose esters

Entry	Compound	CMC (mmol/L)	γ_{CMC} (mN/m)
1	 **1α**	**0.16**	**43.57**
2	 **1β**	**0.17**	**45.19**
3	 **2α**	**0.1**	**52,15**
4	 **2β**	**-**	**-**
5	 **3α** [14]	**0.021**	**49**
6	 **3β** [14]	**0.013**	**47.6**
7	 **4α** [14]	**3.7. 10^{-5}**	**55**
8	 **4β** [14]	**4.9. 10^{-5}**	**51**

Emulsifying properties

The paraffin oil-water system was prepared with a surfactant content of 0.25 % (w/v) in the aqueous phase, in order to investigate the effect of the sugar esters on emulsion formation and stabilization [17; 18]. The heights of the emulsion layer were measured at 25 °C for different concentrations [19]. As can be seen from Figure 1, the emulsifying ability of all products and their anomers is more than 70 %. 1-*O*-octadécanoyl-α-

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D-xylopyranose **2α** showed the best emusifying ability compared to its β-anomer **2β** and to the mixture of the anomers **2**. This sugar monoester has the highest emulsification index value from 100% after 24 hours to 90% after 72 hours, while 1-O-octadecanoy-β-D-xylopyranose has lower emulsification capacity due to its low water solubility. Emulsifying power of 1-*O*-dodécanoyl-D-xylopyranose **1**showed that the β-anomer **1β** is the most stable emulsifier compared to its β-anomer **2β**.

This phenomenon was also observed at various concentrations, at 0.50 % (w/v) and at 0.10 % (w/v). At a concentration of 0.25 % (w/v), the emulsion power of xylose monoesters is most significant. These results indicated that an increase in the length of the lipophilic chain increases the stability of the monoester emulsion because of its compatibility with the parafine oil-water system [19].

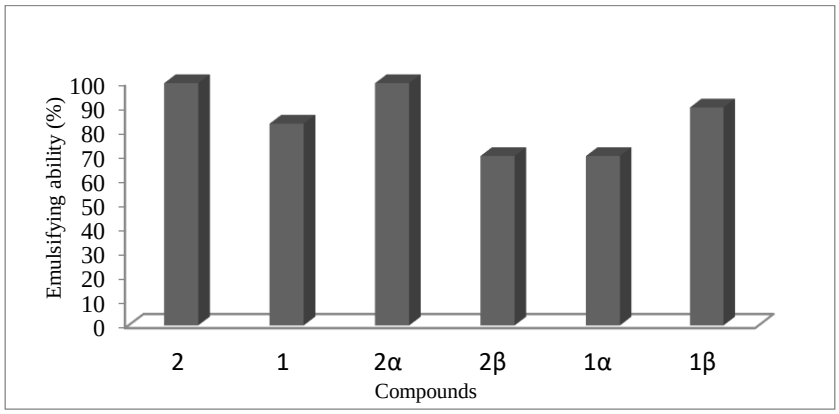

Fig. 1. Emulsifying ability of 0.25 wt % aqueous solutions at 25 °C of xylose esters

The emulsion stability as a function of time for different esters is shown in Figure 2. The tested esters have excellent emulsion properties. Their stability decreased with increasing time, which was clearly seen at a concentration of 0.25% (w/v). A comparative study of the stability of the emulsifier formed by the synthesized products and their anomers shows that for compound **2** 1-*O*-octadecanoyl-α-D-xylopyanose **2α** is the most stable anomer after 72 hours. No stability is observed after two hours for the β-anomer **2β**. For compound **1,** 1-*O*-dodecanoyl-β-D-xylopyranose **1β** is still stable than the α-anomer **1α** and the mixture of the two anomers **2** after 48 and 72 hours as revealed in Figure 2.

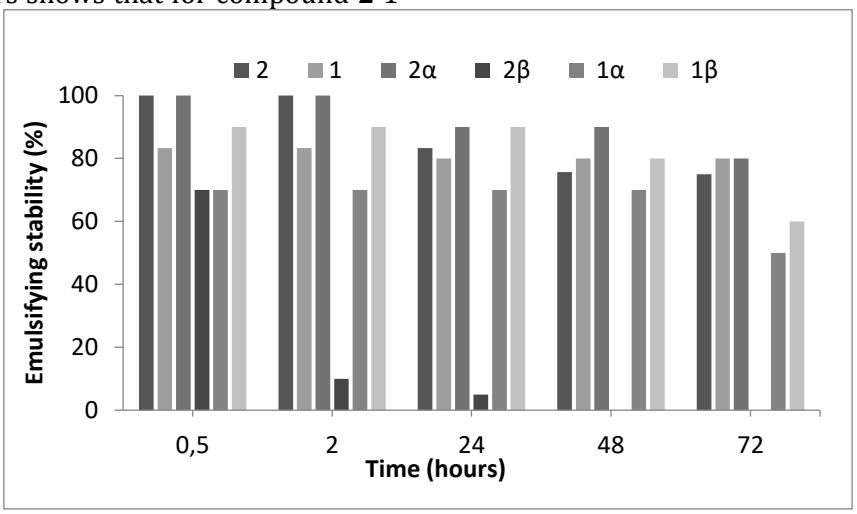

Fig. 2. Emulsifying stability of 0.25 wt % aqueous solutions at 25 °C of xylose esters

Density Functional Theory study

Gaussian 09 software [20] and DFT [21] modeling method were applied to determine the spatial conformation of the synthesized anomeric xylose esters. We have tested tow basis set 3.21 G and 6.31 G of B3LYP, the most famous hybrid density functional theory model. The most stable optimized geometry was obtained from

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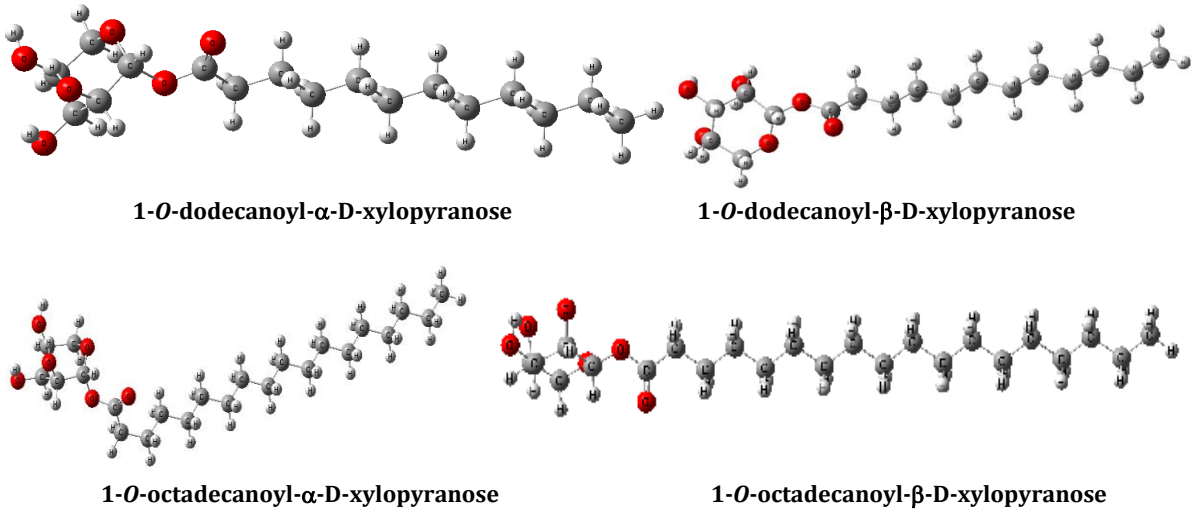
B3LYP/6-31G with the basis set combination 6-31 G (d, p) and 6-31 G (d) method [22]. The atom numbering schemes of the molecules are shown in Figure 3.



1-*O*-dodecanoyl-α-D-xylopyranose **1-*O*-dodecanoyl-β-D-xylopyranose**

1-*O*-octadecanoyl-α-D-xylopyranose **1-*O*-octadecanoyl-β-D-xylopyranose**

Fig. 3. Optimized molecular structure of xylose esters by Gaussian 9 Basis-set 6.31G

The frontier molecular orbitals HOMO and LUMO are the orbitals that are involved in the reactivity. The lowest unoccupied molecular orbital (LUMO) and the highest occupied molecular orbital (HOMO) are located over the whole skeleton of the molecule except the methyl groups of the hydrophobic chain (Figure 3). Their energy value and their energy gaps reflect the reactivity of the molecule. A molecule having a small frontier orbitals gap is a soft molecule with a high chemical reactivity. While a large HOMO-LUMO gap indicates a hard molecule, more stable /less reactive molecule. As presented in Table 2, the compound which have the lowest energetic gap is 1-*O*-octadecanoyl-α-D-xylopyranose (ΔE_{gap} = 0.26237 eV). The results of energy gap indicate that the α-anomers of the two products 1-*O*-dodecanoyl-D-xylopyranose **1α** (ΔE_{gap} = 0.26294 eV) and 1-*O*-octadecanoyl-D-xylopyranose **2α** (ΔE_{gap} = 0.26237 eV) are the softest compounds compared to their β-anomers (1-*O*-dodecanoyl-β-D-xylopyranose, ΔE_{gap} = 0.26315 and 1-*O*-octadecanoyl-β-D-xylopyranose, ΔE_{gap} = 0.26311). According to these results (Table 2), the α-acylxylopyranoses are more reactive than the β ones.

Table 2

Calculated energy values using B3LYP/6-31G basis set

Copmpund	HOMO	LUMO	Energy gap (ev)
1α	–0.25801	0.00493	0.26294
1β	–0.25548	0.00767	0.26315
2α	–0.25866	0.00371	0.26237
2β	–0.25548	0.00763	0.26311

Antibacterial Activity

The antibacterial activity of the synthesized α- and β-anomers of xylose fatty acid ester compounds was tested against five common food-related bacteria including *Staphylococcus aureus* (Gram-positive bacterium), *Bacillus subtilis (*Gram-positive bacterium), *Escherichia coli (*Gram-negative bacterium), *Pseudomonas aeruginosa* (Gram-negative bacterium) and *Klebsiella pneumonia (*Gram-negative bacterium) using the paper disk diffusion assay [23] described by Kirby-Bauer. Filter discs impregnated with 20 µl of DMSO were employed as a negative control, and standard discs containing the antibacterial agent gentamicin were used as control for antibacterial activity.

The antibacterial activities against the five bacteria was evaluated by measuring the zone inhibition growth diameters (ZOI). Table 3 shows the antimicrobial properties of the two anomers of compounds **1α** and **1β**. The results indicate that both anomers have antibacterial activity against all the tested bacteria (Gram-positive and Gram-negative). The inhibition zones obtained are in the range of 16.51–19.86 mm. The tested bacteria are sensitive to the both products (ZOI ≥ 12) [24].

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Table 3

Inhibition zone diameter of tested bacteria strains using 1α and 1β anomers

	Inhibition zone diameter (mm)		
Bacteria strain	**1α**	**1β**	ATB Control[a]
Staphylococcus aureus (+)	16.51 ± 0.74	14.22 ± 0.65	25.17 ± 0.23
Bacillus subtilis (+)	19.86 ± 1.16	17.38 ± 1.24	27.31 ± 1.19
Escherichia coli (-)	17.43 ± 0.82	16.13 ± 0.78	29.76 ± 1.94
Pseudomonas aeruginosa (-)	16.86 ± 0.79	15.03 ± 0.81	26.28 ± 0.86
Klebsiella pneumonia (-)	16.55 ± 1.14	14.95 ± 1.05	28.34 ± 2.3

[a]*ATB Control: Gentamicin*

The antimicrobial test of compounds **2α** and **2β** display also an antibacterial activity against the same bacteria strains (Table 4) and the obtained ZOI are in the range of 11.51–15.16 (≥ 12).

Table 4

Inhibition zone diameter of tested bacteria strains using 2α and 2β anomers

	Inhibition zone diameter (mm)		
Bacteria strain	**2α**	**2β**	ATB Control[a]
Staphylococcus aureus (+)	14.34 ± 0.42	13.18 ± 0.54	24.12 ± 0.18
Bacillus subtilis (+)	15.16 ± 0.92	14.26 ± 0.49	26.25 ± 1.04
Escherichia coli(-)	13.25 ± 0.66	13.33 ± 0.64	30.19 ± 1.67
Pseudomonas aeruginosa (-)	11.51 ± 0.73	12.15 ± 0.52	26.89 ± 0.76
Klebsiella pneumonia (-)	14.75 ± 0.84	13.81 ± 0.29	26.94 ± 1.97

[a]*ATB Control: Gentamicin*

Depending on the fatty acid length, we observed different inhibitory effects on bacterial growth. The **1α** and **1β** anomers of 1-*O*-dodecanoyl-D-xylopyranose (C12) showed the best antimicrobial activity compared to the **2α** and **2β** anomers of 1-*O*-octadecanoyl-D-xylopyranose (C18) implying that increasing the chain length of the fatty acid decreases antimicrobial activity of the considered sugar fatty acid ester. Similar results have been reported in the literature that medium chain length of sugar esters have higher activity than long chain ones [25].

Furthermore, from Tables 2 and 3 and for all bacteria tested, the results showed that the α-anomer exhibited better activity than the β-anomer, especially against *Bacillus subtilis* and *Escherichia coli* with the inhibition zone diameters of 19.86 ± 1.16 and 17.43 ± 0.82 respectively for **1α** ; *Bacillus subtilis* and *Klebsiella pneumonia* with the inhibition zone diameters of 15.16 ± 0.92 and 14.75 ± 0.84 respectively for **2α**.

Minimum inhibitory concentrations (MIC)

The MICs of anomers of xylose esters against the tested bacteria are determined and shown in Tables 5 and 6. The anomer **1α** showed minimum inhibitory concentrations MIC values of 4 and 8 μg/ml against respectively *Escherichia coli* and *Klebsiella pneumonia* (Table 5). These values are close to the MIC obtained by the control antibiotic Gentamicin (1 μg/ml).For compound **2α** the lowest values of minimum inhibitory concentrations MIC are 16 μg/ml against and *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Table 6).

The α anomer is not only more active than the β-anomer, but also acts at a lower concentration. For example, we need 8 μg/ml of **1α** anomer to inhibit the growth of *Klebsiella pneumonia* and 32 μg/ml to inhibit the same bacteria by **1β** anomer.

From these results, it can also be concluded that, in general, the MIC antibacterial activity depends on the structure of the sugar ester molecule and increases with increasing chain length of the fatty acid.

Table 5

Minimum inhibitory concentration (MIC) of 1α and 1β anomers

	MIC (μg/ml)		
Bacteria Strain	**1α**	**1β**	Gentamicin
Staphylococcus aureus	32	64	0.5
Bacillus subtilis	64	128	8
Escherichia coli	4	32	1
Pseudomonas aeruginosa	16	128	4
Klebsiella pneumonia	8	32	2

Table 6

Minimum inhibitory concentration (MIC) of 2α and 2β anomers

	MIC (µg/ml)		
Bacteria Strain	**2α**	**2β**	Gentamicin
Staphylococcus aureus	64	64	0.5
Bacillus subtilis	64	128	8
Escherichia coli	32	32	1
Pseudomonas aeruginosa	16	32	4
Klebsiella pneumonia	16	64	2

Conclusion

We report herein the preparation of the α- and β-anomers of 1-*O*-dodecanoyl-D-xylanopyranose and 1-*O*-octadecanoyl-D-xylanopyranose by acylation and then deacetylation of the corresponding acetylated xylose esters.

Surface tension measurements were used to determine the CMC of the obtained anomeric forms, where the bio-based surfactants show difference in their efficiency in reducing the surface tension of water. The present study also revealed that the incorporation of carbonyl group in xylopyranoside esters skeleton enhances their surface active properties. Oil-water emulsifying ability and emulsion stability of the monoesters were measured. The results indicated that the surface properties were affected by the length and nature of carbon chain. In addition, the separated anomers of sugar esters with different fatty acid moieties displayed different degrees of antibacterial activity against the different bacterial strains tested.

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