Carbohydrate Polymers 119 (2015) 1–7

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

One-pot synthesis of thermoplastic mixed paramylon esters using trifluoroacetic anhydride

Motonari Shibakami [a,*], Gen Tsubouchi [a], Mitsugu Sohma [b], Masahiro Hayashi [c]

[a] Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Central 6th, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan
[b] Advanced Manufacturing Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Central 5th, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8565, Japan
[c] Department of Marine Biology and Environmental Science, Faculty of Agriculture, University of Miyazaki, 1-1 Gakuen Kibanadai-nishi, Miyazaki 889-2192, Japan

ARTICLE INFO

Article history:
Received 20 September 2014
Received in revised form
11 November 2014
Accepted 13 November 2014
Available online 24 November 2014

Keywords:
Thermoplastic
Paramylon
One-pot synthesis
Esterification
Euglena

ABSTRACT

Mixed paramylon esters prepared from paramylon (a storage polysaccharide of *Euglena*), acetic acid, and a long-chain fatty acid by one-pot synthesis using trifluoroacetic anhydride as a promoter and solvent were shown to have thermoplasticity. Size exclusion chromatography indicated that the mixed paramylon esters had a weight average molecular weight of approximately $4.9 - 6.7 \times 10^5$. Thermal analysis showed that these esters were stable in terms of the glass transition temperature (>90 °C) and 5% weight loss temperature (>320 °C). The degree of substitution of the long alkyl chain group, a dominant factor determining thermoplasticity, was controlled by tuning the feed molar ratio of acetic acid and long-chain fatty acid to paramylon. These results implied that the one-pot synthesis is useful for preparing structurally-well defined thermoplastic mixed paramylon esters with high molecular weight.

1. Introduction

The creation of products from microalgae has recently attracted particular attention, mainly because of their ability to fix carbon dioxide and metabolize carbon sources in wastewater (Chisti, 2007). The target products that have been mostly studied are biofuels with the expectation of reducing dependency on fossil fuel (Banerjee, Sharma, Chisti, & Banerjee, 2002; Kaya et al., 2011; Mata, Martins, & Caetano, 2010; Tucci, Vacula, Krajcovic, Proksch, & Martin, 2010; Yamane et al., 2013). Despite intensive efforts to make the production more efficient, further developments for reducing production cost are required for practical use.

Our efforts in this area have focused on the creation of high-value-added organic materials from polysaccharide of *Euglena* (Shibakami, Sohma, & Hayashi, 2012; Shibakami, Tsubouchi, Nakamura, & Hayashi, 2013). This polymer, which is referred to as paramylon, is a storage polysaccharide in which glucose units are linked by β-1,3-bonds (Barras & Stone, 1968). Since paramylon is produced in significant quantities by using readily cultured euglenoid alga (Barsanti, Vismara, Passarelli, & Gualtieri, 2001; Santek, Friehs, Lotz, & Flaschel, 2012), this polymer is a promising biomass. We recently reported the creation of *Euglena*-based thermoplastics from mixed paramylon esters (Shibakami, Tsubouchi, & Hayashi, 2014). The procedure we used for synthesizing these esters is two-pot esterification in which a long alkyl chain and acetyl groups are sequentially introduced into the glucose unit of paramylon. Although this two-pot synthetic procedure is not complicated, the development of a one-pot synthetic method may lead to *Euglena*-based thermoplastics becoming more practical.

Our ultimate goal is to establish a one-pot method for synthesizing thermoplastic mixed paramylon esters as a component of *mechanically* and *thermally* stable *Euglena*-based plastics. The immediate objective of the work reported here was to find a one-pot synthetic method that produces paramylon with thermoplasticity. In particular, we examined the feasibility of one-pot esterification using trifluoroacetic anhydride (TFAA). While the preparation of several polysaccharide esters using this one-pot synthesis has been reported (Marubayashi, Yukinaka, Enomoto-Rogers, Takemura, & Iwata, 2014; Yang & Montgomery, 2008; Yang, Ding, & Montgomery, 2009), there has been no study of paramylon esters. In this paper, we describe the suitability of one-pot esterification for producing thermoplastic mixed paramylon esters

* Corresponding author. Tel.: +81 29 861 4547; fax: +81 29 861 4547.
E-mail address: moto.shibakami@aist.go.jp (M. Shibakami).

http://dx.doi.org/10.1016/j.carbpol.2014.11.036
0144-8617/

comparable to those prepared by two-pot esterification. We also briefly compare the structural and thermal properties of mixed paramylon esters prepared using one-pot and two-pot synthesis.

2. Experimental

2.1. General methods

^{1}H nuclear magnetic resonance (NMR) spectra were recorded on Bruker AVANCE 500 and JEOL JNM-ECX400 spectrometers. Quantitative ^{13}CNMR spectrum was obtained using the inverse-gated decoupling method (65,536 cumulative times). Fourier transform infrared (FT-IR) spectra were recorded using a JASCO FT/IR-480ST spectrophotometer equipped with an attenuated total reflectance accessory (ATR Pro 400-S, ZnSe prism, JASCO) with a resolution of 4 cm^{-1}. The melting behavior of the products was observed using a Yanako MP-500D melting-point apparatus. Optical microscopic observation was performed with a Leica DMRE microscope.

All chemicals and reagents were used without further purification. Paramylon particles were obtained from *Euglena gracilis* in accordance with a previously reported method (Shibakami et al., 2012). The degree of substitution (DS) values, i.e., the average number of functional groups attached to a glucose unit, were determined by comparing the integral values of the methyl protons of the long alkyl chain and acetyl group with those of the glucosidic protons in the ^{1}HNMR spectrum. The calculation of DS values takes account of the non-functionalized OH groups that have signals appearing in the glucosidic proton range. The equations used for the calculation were as follows.

$$3DS_{ace} \times \frac{7.0}{7.0 + DS_H} = a$$

$$3DS_{lac} \times \frac{7.0}{7.0 + DS_H} = b$$

$$DS_{ace} + DS_{lac} + DS_H = 3.0$$

where DS_{ace}, DS_{lac}, and DS_H represent the DS of acetyl, long alkyl chain (octanoyl, decanoyl, lauroyl, myristoyl, palmitoyl, or stearoyl), and proton, respectively; a and b are the relative integration value due to the methyl group of acetyl and long-chain alkyl group, respectively, when the total glucosidic protons were set to 7.0.

2.2. Synthesis of mixed paramylon esters

2.2.1. Paramylon acetate myristate prepared using one-pot synthesis in DMAc/LiCl from paramylon, acetic anhydride, and myristoyl chloride (**1**)

To a homogeneous solution of paramylon (10.00 g, 61.68 mmol) and lithium chloride (LiCl) (7.84 g, 185.00 mmol) in N,N-dimethylacetamide (DMAc) (500 mL) prepared by heating at ~120 °C for 0.5 h were added dropwise at room temperature triethylamine (NEt_3) (43.3 mL, 308.51 mmol) and a solution made from DMAc (500 mL) and myristoyl chloride (8.4 mL, 30.84 mmol). This mixture was heated at 120 °C under a nitrogen atmosphere for 3 h and then cooled to 70 °C. Next, NEt_3 (300 mL, 2.15 mmol), acetic anhydride (240 mL, 2.54 mmol), and DMAc (500 mL) were added to the solution. After mechanical stirring at 70 °C for 5 h and at room temperature for 14 h, a mixture of methanol (1500 mL) and water (140 mL) was added to precipitate a solid. After removal of supernatant by filtration, the solid was washed with water (1600 mL) by mechanical stirring for 1.5 h. Air-drying for 4 h and subsequent vacuum-drying at 90 °C for 1 h produced a dark orange-colored solid (20.59 g). A part of this solid was purified as follows. To a solution made from the solid (1.03 g) and chloroform (25 mL) was added methanol (250 mL) to precipitate a yellowish solid. This procedure was repeated two times. Air-drying for 16 h and subsequent vacuum-drying at 90 °C for 2 h produced paramylon acetate myristate (**1**) as a pale yellowish solid (734 mg, 2.62 mmol, yield 84.3%). ^{1}HNMR ($CDCl_3$) δ 5.04–4.71(m), 4.53–4.13(m), 4.11–3.96(m), 3.87–3.33(m), 2.47–2.33(m), 2.12(s), 2.06(s), 2.00(s), 1.60(m), 1.26(s), 0.88 (t, $J \equiv 6.8$). FT-IR (cm^{-1}) 2925, 2852, 1742, 1636, 1434, 1368, 1213, 1190, 1119, 1037, 889.

2.2.2. Paramylon acetate myristate prepared using one-pot synthesis and TFAA from paramylon (1 eq), acetic acid (10 eq), and myristic acid (6 eq) (**2**)

To a round-bottomed flask containing TFAA (8.0 mL) were added acetic acid (710 μL, 12.41 mmol) and myristic acid (1.499 g, 7.48 mmol) at 50 °C. After stirring for 5 min, paramylon (200 mg, 1.23 mmol) was added to the solution under a nitrogen atmosphere and stirred for 1 h. The resulting homogeneous solution was poured into a saturated sodium hydrogen carbonate aqueous solution (400 mL). A white solid was obtained from this solution by filtration. The solid was washed with water (80 mL) for 15 min and methanol (80 mL) for 15 min by mechanical stirring (two times), followed by vacuum-drying at 90 °C for 2 h to yield paramylon acetate myristate (**2**) (523 mg, 1.15 mmol, yield 93.5%). ^{1}HNMR ($CDCl_3$) δ 4.95–4.65(m), 4.43–4.14(m), 4.07–3.91(m), 3.81–3.52(m), 2.41–2.21(m), 2.12(s), 2.06(s), 2.00(s), 1.59(m), 1.26(s), 0.88 (t, $J \equiv 6.6$). FT-IR (cm^{-1}) 2918, 2851, 1745, 1456, 1368, 1213, 1146, 1111, 1038, 1026, 890, 799, 721, 631.

2.2.3. Paramylon acetate myristates prepared using one-pot synthesis and TFAA from paramylon, acetic acid, and myristic acid with different feed molar ratio (**3**–**5**)

Procedures similar to those described for the preparation of **2** were used to obtain paramylon acetate myristate (**3**–**5**) using different feed molar ratio (i.e., 1:11:5, 1:12:4, and 1:14:2 (paramylon (glucose unit): acetic acid: long-chain fatty acid)). Yields (%) were 78.1 (**3**), 71.4 (**4**), and 72.7 (**5**).

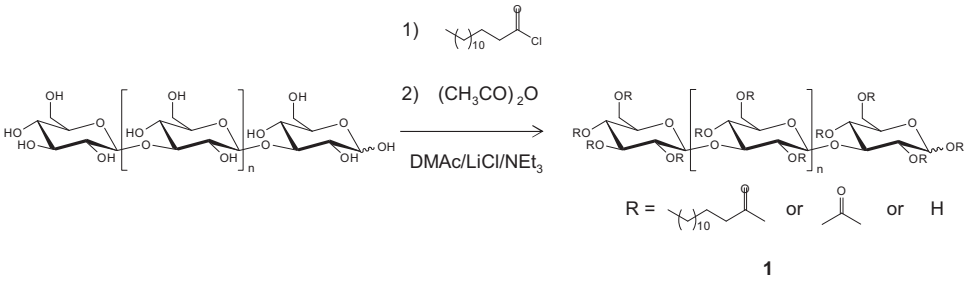
2.2.4. Mixed paramylon esters prepared using one-pot synthesis and TFAA from paramylon (1 eq), acetic acid (12 eq), and myristic acid (4 eq) (**6**–**10**)

Procedures similar to those described for the preparation of **2** were used to obtain mixed paramylon esters (**6**–**10**) made from paramylon, acetic acid, and different long-chain fatty acid (octanoic acid, decanoic acid, lauric acid, palmitic acid, and stearic acid) with a feed molar ratio of 1:12:4. Yields (%) were 92.5 (**6**), 88.4 (**7**), 88.3 (**8**), 91.1 (**9**), and 92.0 (**10**).

2.2.5. Paramylon acetate myristate prepared using two-pot synthesis in DMAc/LiCl from paramylon, acetic anhydride, and myristoyl chloride (**11**)

Two-pot synthesis of paramylon acetate myristate was done using a method previously reported (Shibakami et al., 2014). To a homogeneous solution of paramylon (10.00 g, 61.68 mmol) and LiCl (7.84 g, 185.00 mmol) in DMAc (500 mL) prepared by heating at ~120 °C for 0.5 h were added dropwise at room temperature NEt_3 (43 mL, 308.38 mmol) and a solution made from DMAc (500 mL) and myristoyl chloride (8.4 mL, 30.9 mmol). This mixture was heated at 120 °C under a nitrogen atmosphere for 3 h. Methanol (1200 mL) was then added to precipitate a solid. After removal of supernatant by centrifugation, the solid was washed with a mixture of methanol (600 mL) and chloroform (300 mL) for 1 h. The wet solid separated by centrifugation was air-dried for 15 h and subsequently vacuum-dried at 90 °C for 2 h to produce paramylon myristate as a solid (15.00 g).

To a homogeneous solution containing DMAc (1500 mL), LiCl (6.50 g, 153.46 mmol), and paramylon myristate (15.00 g), which



Scheme 1. Scheme for synthesizing paramylon acetate myristate from paramylon, acetic anhydride, and myristoyl chloride using one-pot synthesis in DMAc/LiCl solvent system.

was prepared by heating at ~120 °C for 0.5 h, were added pyridine (168 mL, 2.09 mol) and acetic anhydride (240 mL, 4.89 mol) at 70 °C. The mixture was heated at 70 °C for 5 h and then left at ambient temperature for 17 h under a nitrogen atmosphere. Addition of water (3000 mL) to the mixture produced a solid. This solid was washed with methanol (1600 mL) for 15 min by mechanical stirring. After separation by filtration, the resulting solid was air-dried for 17 h and subsequently dried under reduced pressure at 90 °C for 7 h to produce paramylon acetate myristate (**11**) as a white solid (16.10 g, 48.06 mmol, yield 77.9%). ^{1}HNMR ($CDCl_3$) δ 5.09–4.69(m), 4.49–4.16(m), 4.11–3.90(m), 3.82–3.47(m), 2.47–2.21(m), 2.12(s), 2.06(s), 1.99(s), 1.60(m), 1.26(s), 0.88 (t, $J \equiv 6.7$). FT-IR (cm^{-1}) 2925, 2852, 1742, 1630, 1434, 1368, 1213, 1167, 1119, 1037, 889.

2.3. Size exclusion chromatography

The weight average molecular weights (M_w) of mixed paramylon esters were determined by using size exclusion chromatography with multiangle laser light scattering (SEC-MALLS). The SEC-MALLS measurements were carried out on a DAWN HELEOS II multiangle laser photometer (Wyatt Technology) and an Optilab T-rEX refractive index detector (Wyatt Technology) equipped with a Shodex KD-805 gel permeation chromatography column (eluent chloroform, 1.0 mL/min, 40 °C). All of the polysaccharide solutions were purified using a 0.2-μm filter prior to injection. The injection volume was 100 μL with a concentration of approximately 4.0 mg/mL. The dn/dc value was 0.0403.

2.4. Differential scanning calorimetry and thermogravimetric analysis

Differential scanning calorimetrry (DSC) were carried out with a Rigaku Thermo plus EVO II DSC8230 calorimeter. The sample was heated from 25 to 230 °C at a scan rate of 10.0 °C/min and held at 230 °C for 3 min. After cooling from 230 to 25 °C and holding for 5 min at this temperature, the sample was heated to 250 °C at the same scan rate. Thermograms obtained during the second heating were used for determining the glass transition temperature (T_g). Thermogravimetric (TG) analyses were carried out using a Rigaku Thermo plus EVO II TG8120 thermogravimetric analyzer. The sample was heated from 25 to 500 °C at a heating rate of 10.0 °C/min under a nitrogen flow of 100 mL/min.

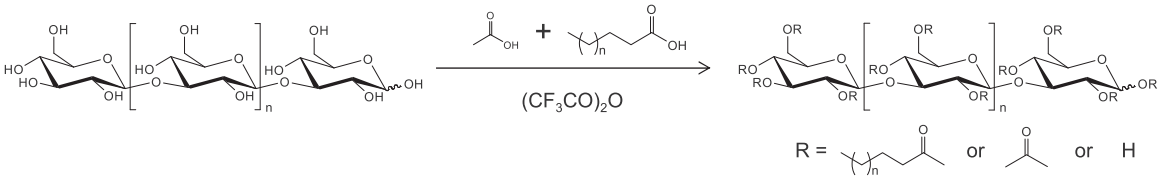
2.5. X-ray diffraction measurement

X-ray diffraction (XRD) diagrams were recorded using a MAC Science MFX-HP diffractometer with monochromatic Cu-Kα radiation ($\lambda \equiv 0.15418$ nm) generated at 40 kV and 30 mA through an optical slit system: divergence slit ≡ 1°; scattering slit ≡ 1°; and receiving slit ≡ 0.3 mm. Scattering was performed as: scattering angle, $2\theta \equiv 3$–35° with 2θ step of 0.05° and accumulation time of 5 s.

3. Results and discussion

3.1. Exploration of feasibility of one-pot synthesis in DMAc/LiCl as a means for producing mixed paramylon esters

First, we examined the feasibility of one-pot esterification modified from the two-pot synthesis previously reported (Shibakami et al., 2014). In brief, myristoyl chloride and acetic anhydride were sequentially added to a homogeneous DMAc/LiCl solution containing paramylon without purifying the intermediate products (Scheme 1). A naked-eye observation of the melting behavior of the resulting solid (**1**) on a hot plate revealed that it did not show



		acetic acid/myristic acid
2	n = 10	10/6
3	n = 10	11/5
4	n = 10	12/4
5	n = 10	14/2
		acetic acid/long-chain fatty acid
6	n = 4	12/4
7	n = 6	12/4
8	n = 8	12/4
9	n = 12	12/4
10	n = 14	12/4

Scheme 2. Scheme for synthesizing mixed paramylon esters from paramylon, acetic acid, and long-chain fatty acid using one-pot synthesis and TFAA.

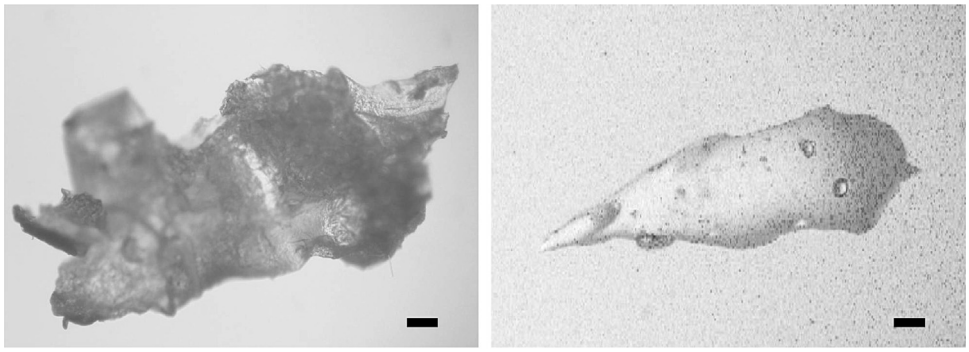


Fig. 1. Optical microscopic images of paramylon acetate myristate (**2**): (a) before and (b) after heating to 230 °C. Bars represent 0.1 mm.

thermoplasticity, even upon heating up to 300 °C. This result indicated that this one-pot synthesis cannot produce thermoplastic mixed paramylon esters. To examine the reason for the absence of thermoplasticity, we carried out ^{1}HNMR measurement on **1**. The spectrum showed that DS_{ace} and DS_{lac} were 2.58 and 0.07, respectively. This DS_{lac} value is too low for the feed molar ratio of myristoyl chloride to paramylon (glucose unit) of 0.5 eq. Since thermoplastic mixed paramylon esters have a DS_{lac} of above ca. 0.3 (Shibakami et al., 2014), it is plausible that the absence of thermoplasticity was due to a low DS_{lac} value.

3.2. Exploration of feasibility of one-pot synthesis using TFAA as a means for producing mixed paramylon esters

Next, we examined the feasibility of one-pot synthesis using TFAA as a promoter and solvent. To examine the feasibility of this synthetic method for preparing thermoplastic mixed paramylon esters, we attempted to synthesize paramylon acetate myristate using procedures similar to those previously reported (Marubayashi et al., 2014). In brief, to a flask containing mixed anhydrides made from TFAA, acetic acid, and myristic acid was added paramylon at a feed molar ratio of 1:10:6 (paramylon (glucose unit):acetic acid: myristic acid) (Scheme 2). The heterogeneous solution containing paramylon and acids became homogeneous within 20 min upon addition of paramylon, which indicates the progress of acylation. Subsequent addition of the reaction mixture to a sodium hydrogen carbonate aqueous solution yielded the desired product (**2**) as a white solid. The ^{1}HNMR spectrum of **2** confirmed successful introductions of both acetyl and a long alkyl chain group into the paramylon, with DS_{ace} and DS_{lac} values of 1.84 and 1.05, respectively. A naked-eye observation of the melting behavior of **2** on a hot plate revealed that this compound had thermoplasticity (Fig. 1). These results demonstrate that TFAA-based one-pot synthesis can be used to prepare thermoplastic mixed paramylon esters.

3.3. One-pot synthesis using TFAA of paramylon acetate myristates from paramylon, acetic acid, and myristic acid with different feed molar ratios

The DS_{lac} of mixed polysaccharide esters is a dominant factor determining thermoplasticity (Iji, Toyama, & Tanaka, 2013; Shibakami et al., 2014; Takihara, Yoshida, & Isogai, 2007; Yoshioka, Hagiwara, & Shiraishi, 1999). The feasibility of controlling the DS values by changing the feed molar ratio of the acids has been demonstrated for preparation of mixed cellulose esters (Yang & Montgomery, 2008). To examine the applicability of this method to the control of the DS values of mixed paramylon esters, we synthesized paramylon acetate myristates using different feed molar ratios. The total feed molar equivalent of acids to paramylon (glucose unit) was fixed at 16 eq.

The DS_{total}, DS_{ace}, and DS_{lac} values of the synthesized paramylon acetate myristates are summarized in Table 1. The DS_{total} values ranged from 2.59 to 2.89, indicating that full acylation was not achieved under the current reaction conditions. Table 1 also shows that DS_{lac} can be controlled by changing the feed molar ratio of the long-chain fatty acid: the smaller the ratio, the smaller the DS_{lac}. The M_w values ranged from approximately 4.9 to 6.7 $\times 10^5$ Da, and the degree of polymerization (DP) ranged from about 1200 to about 1900.

A naked-eye observation of the melting behavior of products **2**–**5** on a hot plate revealed that all the compounds had thermoplasticity. To evaluate their thermal properties quantitatively, we carried out TG and DSC measurements. Thermograms are shown in Figs. 2 and 3. Since no crystallization or melting peak was present in each DSC curve, it is apparent that the mixed esters are glassy and amorphous at room temperature, which was confirmed also by XRD measurement showing amorphous halo (Marubayashi et al., 2014) (see Supplementary data). Thermal property values determined from the thermograms are shown in Table 1. T_g tended to increase with a decrease in DS_{lac}. This is attributed to the contribution of the myristoyl group to the increasing of the intermolecular chain distance. Further examination revealed that the T_g of **2** was higher

Table 1
Degree of substitution, molecular weight, degree of polymerization, and thermal properties of paramylon acetate myristates prepared using different feed ratios.

	Feed molar ratio of paramylon (glucose unit)/acetic acid/myristic acid	DS_{total}	DS_{ace}	DS_{lac}	M_w (Da)	DP	T_g (°C)	T*d*5 (°C)
2	1/10/6	2.89	1.84	1.05	540,500	1187	96.6	330.1
3	1/11/5	2.85	2.15	0.70	559,700	1414	93.4	351.4
4	1/12/4	2.69	2.14	0.55	673,600	1872	106.2	345.8
5	1/14/2	2.59	2.24	0.35	496,000	1547	127.0	339.8

Table 2
Degree of substitution, molecular weight, degree of polymerization, and thermal properties of mixed paramylon esters made from paramylon, acetic acid, and long-chain fatty acid with a feed molar ratio of 1:12:4.

	Long alkyl chain	DS_{total}	DS_{ace}	DS_{lac}	M_w (Da)	DP	T_g (°C)	T*d*5 (°C)
6	Octanoyl	2.68	1.95	0.73	581,600	1767	107.2	337.1
7	Decanoyl	2.73	2.04	0.69	536,900	1550	106.8	337.1
8	Lauroyl	2.70	2.03	0.67	582,900	1616	105.1	339.7
9	Palmitoyl	2.69	2.08	0.61	559,400	1457	101.3	321.3
10	Stearoyl	2.71	2.20	0.51	661,800	1735	106.4	322.2

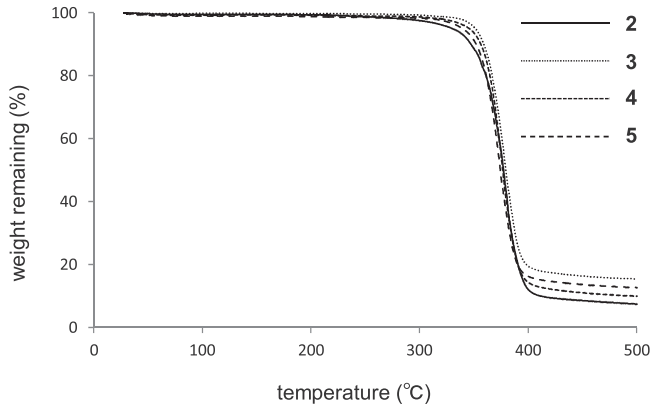


Fig. 2. TG curves of paramylon acetate myristates (**2**–**5**).

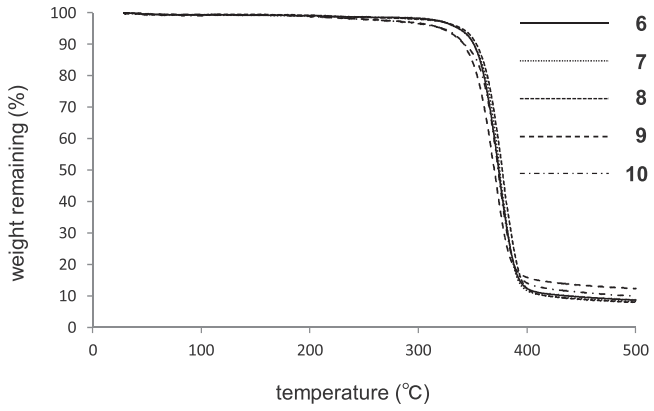


Fig. 4. TG curves of mixed paramylon esters (**6**–**10**).

than that of **3** although the DS_{lac} of **2** was substantially higher than that of **3**. This indicates that the introduction of a long-chain alkyl group with a high DS_{lac} may induce an interchain interaction that suppresses molecular fluctuation. As for the 5% weight loss temperature (T*d*5), while there was no apparent relationship between this parameter and DS_{lac}, **2**–**5** did show a higher T*d*5 (>330 °C) than paramylon (ca. 300 °C).

3.4. One-pot syntheses using TFAA of mixed paramylon esters from paramylon, acetic acid, and long-chain fatty acid having different alkyl chain lengths

To examine how the alkyl chain length of the long-chain fatty acid affects the polymeric structure and thermal properties of the mixed paramylon esters, we synthesized esters having octanoyl, decanoyl, lauroyl, palmitoyl, or stearoyl as the long-chain alkyl group using a feed molar ratio of 1:12:4 (paramylon (glucose unit):acetic acid:long-chain fatty acid) (Scheme 2).

Table 2 summarizes the structural parameters and thermal properties of the products (**6**–**10**). The DS_{total} values ranged from 2.68 to 2.73. That the DS_{total} of **4** was 2.69 indicates that the DS_{total} value is nearly constant despite the differences in the alkyl chain length of the long-chain fatty acids if the total feed molar equivalent of the acids is fixed. Table 2 also shows that DS_{lac} tended to decrease with an increase in the long alkyl chain length although the DS_{lac} of **4** (myristoyl) was between that of **9** (palmitoyl) and **10** (stearoyl). This indicates that the efficacy of long alkyl chain group introduction depends on its chain length.

All the molecular weight values for **4** and **6**–**10** were above 5.3×10^5 Da, and the DP values ranged from 1457 to 1872. If the paramylon used in this study had M_w and DP values similar to those previously reported (M_w of 2.959×10^5 Da, DP of 1827) (Shibakami et al., 2013), it is likely that depolymerization barely occurred with the current synthetic procedures. A naked-eye observation of the melting behavior of **6**–**10** showed that all of them had thermoplasticity as expected. Figs. 4 and 5 show TG and DSC thermograms indicating that all the T_g and T*d*5 values were above 100 and 320 °C, respectively. Although these thermal properties plausibly depend on the long alkyl chain length, comparison of them among **4** and **6**–**10** is inappropriate because of the different DS_{lac} values.

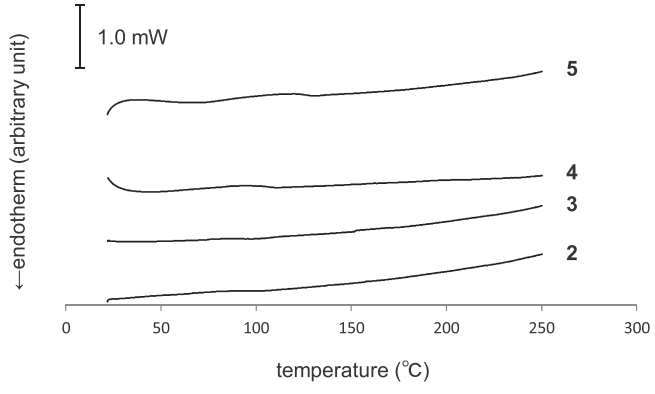


Fig. 3. DSC curves of paramylon acetate myristates (**2**–**5**).

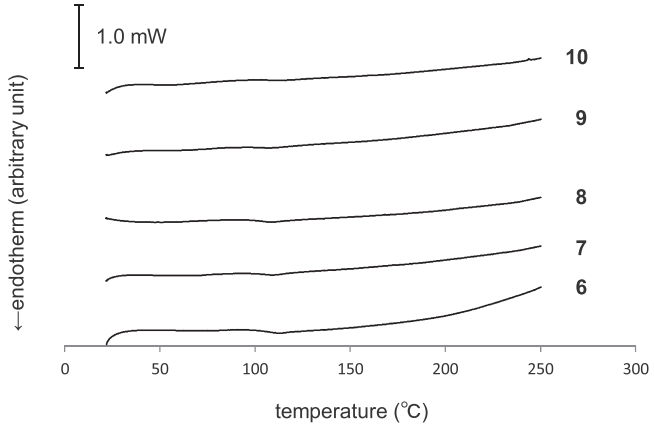


Fig. 5. DSC curves of mixed paramylon esters (**6**–**10**).

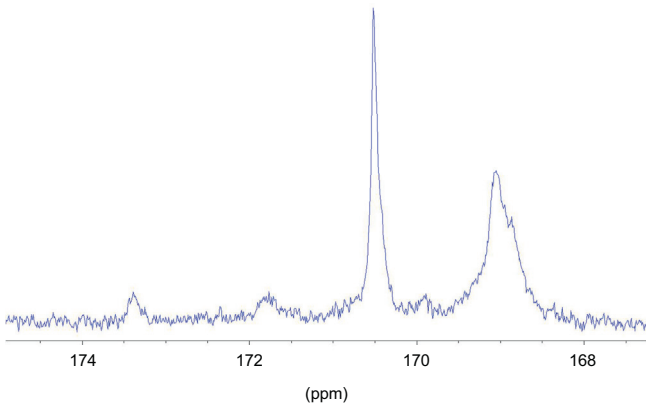


Fig. 6. ^{13}CNMR spectrum (quantitative mode) of paramylon acetate myristate (**5**).

3.5. Comparison of paramylon acetate myristate prepared using one-pot synthesis and TFAA with that prepared using two-pot synthesis in DMAc/LiCl

One additional concern was whether mixed paramylon esters prepared using the one-pot synthesis and TFAA are structurally and thermally comparable to those prepared using the two-pot synthesis in the DMAc/LiCl solvent system previously reported (Shibakami et al., 2014). To examine this, we prepared paramylon acetate myristate (**11**) using the two-pot synthesis. For accurate comparison, we used paramylon from the same lot used for synthesizing **1**–**10**. The ^{1}HNMR showed that the DS_{ace} and DS_{lac} of **11** were 2.35 and 0.38, respectively. Since **5** had the closest DS value to **11**, we compared **5** with **11**. The M_w and DP of **11** were 610,100 Da and 1821, respectively. A hot-plate test confirmed that **11** had thermoplasticity. DSC and TG measurements indicated that the T_g and T*d*5 of **11** were 121.4 and 332.6 °C, respectively. Comparison of these values with those of **5** indicates that the one-pot synthesis produces mixed paramylon esters comparable to those produced by the two-pot synthesis in terms of polymeric structure and thermal properties.

To explore the regioselectivity of acylation using the one-pot synthesis, we carried out quantitative ^{13}CNMR measurement on **5**. A sharp and broad signal appeared at 173.4 and 171.8 ppm. The former was due to myristoyl carbonyl carbon attached to position C6, and the latter was due to its attachment to positions C2 and C4 (C2/C4). Their relative integral values are approximately 1.00 and 1.84, respectively (Fig. 6). Given that the DS_{lac} value of **5** was 0.35, the partial DS_{lac} values of myristoyl at C6 and C2/C4 were 0.12 and 0.23, respectively. In a previous study (Shibakami et al., 2014), we examined the regioselectivity of acylation using the two-pot synthesis by measuring quantitative ^{13}CNMR on paramylon acetate myristate (DS_{lac} 0.48/DS_{ace} 2.24) and found that the partial DS_{lac} values of myristoyl at C6 and C2/C4 were 0.34 and 0.14, respectively. Thus, the one-pot synthesis can produce mixed paramylon esters with DS_{lac} values different from those produced by the two-pot synthesis. Since regiochemistry has a significant influence on the physical, chemical, and thermal properties of polymers, the one-pot synthesis extends the range of application of the thermoplastic mixed paramylon esters in the area of material chemistry.

4. Conclusion

We have demonstrated the feasibility of using one-pot synthesis in the presence of trifluoroacetic anhydride as a promoter and solvent as a means of preparing thermoplastic mixed paramylon esters. The benefits of this method include simple, quick preparation: mixed paramylon esters were obtained within a few hours. An additional benefit is structural controllability: the degree of substitution of acetyl and the long alkyl chain can be controlled by tuning the feed molar ratios of the acetic acid and long-chain fatty acid to paramylon. The mixed paramylon esters produced by the one-pot synthesis were comparable to those produced by two-pot synthesis in the DMAc/LiCl solvent system. Whether the slight difference in the regioselectivity of the long-chain alkylation between the one-pot and two-pot reaction affects the mechanical strength of *Euglena*-based thermoplastics remains to be determined. Studies currently in progress are aimed at answering this question. The results will be reported in due course.

Acknowledgments

This research was partly supported by the Advanced Low Carbon Technology Research and Development Program of the Japan Science and Technology Agency.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.11.036.

References

Banerjee, A., Sharma, R., Chisti, Y., & Banerjee, U. C. (2002). *Botryococcus braunii*: A renewable source of hydrocarbons and other chemicals. *Critical Reviews in Biotechnology*, *22*(3), 245–279. http://dx.doi.org/10.1080/07388550290789513

Barras, D. R., & Stone, B. A. (1968). . *Carbohydrate composition and metabolism* (II) New York: Academic Press.

Barsanti, L., Vismara, R., Passarelli, V., & Gualtieri, P. (2001). Paramylon (beta-1,3-glucan) content in wild type and WZSL mutant of *Euglena gracilis*. Effects of growth conditions. *Journal of Applied Phycology*, *13*(1), 59–65.

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances*, *25*(3), 294–306. http://dx.doi.org/10.1016/j.biotechadv.2007.02.001

Iji, M., Toyama, K., & Tanaka, S. (2013). Mechanical and other characteristics of cellulose ester bonded with modified cardanol from cashew nut shells and additional aliphatic and aromatic components. *Cellulose*, *20*(1), 559–569. http://dx.doi.org/10.1007/s10570-012-9832-z

Kaya, K., Nakazawa, A., Matsuura, H., Honda, D., Inouye, I., & Watanabe, M. M. (2011). Thraustochytrid aurantiochytrium sp 18W-13a accummulates high amounts of squalene. *Bioscience, Biotechnology and Biochemistry*, *75*(11), 2246–2248. http://dx.doi.org/10.1271/Bbb.110430

Marubayashi, H., Yukinaka, K., Enomoto-Rogers, Y., Takemura, A., & Iwata, T. (2014). Curdlan ester derivatives: Synthesis, structure, and properties. *Carbohydrate Polymers*, *103*, 427–433. http://dx.doi.org/10.1016/j.carbpol.2013.12.015

Mata, T. M., Martins, A. A., & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: A review. *Renewable & Sustainable Energy Reviews*, *14*(1), 217–232. http://dx.doi.org/10.1016/j.rser.2009.07.020

Santek, B., Friehs, K., Lotz, M., & Flaschel, E. (2012). Production of paramylon, a ss-1,3-glucan, by heterotrophic growth of *Euglena gracilis* on potato liquor in fed-batch and repeated-batch mode of cultivation. *Engineering in Life Sciences*, *12*(1), 89–94. http://dx.doi.org/10.1002/elsc.201100025

Shibakami, M., Sohma, M., & Hayashi, M. (2012). Fabrication of doughnut-shaped particles from spheroidal paramylon granules of *Euglena gracilis* using acetylation reaction. *Carbohydrate Polymers*, *87*(1), 452–456. http://dx.doi.org/10.1016/j.carbpol.2011.08.012

Shibakami, M., Tsubouchi, G., & Hayashi, M. (2014). Thermoplasticization of euglenoid beta-1,3-glucans by mixed esterification. *Carbohydrate Polymers*, *105*, 90–96. http://dx.doi.org/10.1016/j.carbpol.2014.01.053

Shibakami, M., Tsubouchi, G., Nakamura, M., & Hayashi, M. (2013). Preparation of carboxylic acid-bearing polysaccharide nanofiber made from euglenoid beta-1,3-glucans. *Carbohydrate Polymers*, *98*(1), 95–101. http://dx.doi.org/10.1016/j.carbpol.2013.05.026

Takihara, T., Yoshida, Y., & Isogai, A. (2007). Reactions between cellulose diacetate and alkenylsuccinic anhydrides and characterization of the reaction products. *Cellulose*, *14*(4), 357–366. http://dx.doi.org/10.1007/s10570-007-9112-5

Tucci, S., Vacula, R., Krajcovic, J., Proksch, P., & Martin, W. (2010). Variability of wax ester fermentation in natural and bleached *Euglena gracilis* strains in response to oxygen and the elongase inhibitor flufenacet. *Journal of Eukaryotic Microbiology*, *57*(1), 63–69. http://dx.doi.org/10.1111/j. 1550-7408.2009.00452.x

Yamane, K., Matsuyama, S., Igarashi, K., Utsumi, M., Shiraiwa, Y., & Kuwabara, T. (2013). Anaerobic coculture of microalgae with *Thermosipho globiformans*

and *Methanocaldococcus jannaschii* at 68{degrees}C enhances generation of n-alkane-rich biofuels after pyrolysis. *Applied and Environmental Microbiology*, *79*(3), 924–930. http://dx.doi.org/10.1128/AEM. 01685-12

Yang, B. Y., Ding, Q., & Montgomery, R. (2009). Preparation and physical properties of chitin fatty acids esters. *Carbohydrate Research*, *344*(3), 336–342. http://dx.doi.org/10.1016/j.carres.2008.11.006

Yang, B. Y., & Montgomery, R. (2008). Preparation and physical properties of starch mixed esters. *Starch-Starke*, *60*(3–4), 146–158. http://dx.doi.org/10.1002/star.200700692

Yoshioka, M., Hagiwara, N., & Shiraishi, N. (1999). Thermoplasticization of cellulose acetates by grafting of cyclic esters. *Cellulose*, *6*(3), 193–212. http://dx.doi.org/10.1023/A:1009221730934