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Synthesis and Characterization of Flavonoid Laurate Esters by Transesterification

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ABSTRACT: Laurate esters of catechin and condensed tannins were synthesized by both transesterification and conventional acylation techniques. Transesterification was developed as an alternative route to prepare flavonoid fatty acid esters as a prerequisite to advancing potential applications. Resorcinol, catechin, and two condensed tannins were transesterified with vinyl esters, and the resulting products were compared to those formed with lauroyl chloride. The esterified products, including catechin pentalaurate, were characterized both chemically and thermally. Transesterification produced partially substituted derivatives, with a preference for substitution at the catechol ring. Melt features were identified at or below 50°C, which were dependent upon the level of substitution. In addition, the tannin laurates showed thermal stability to at least 100°C and moderate retention of antioxidant properties. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 129: 181–186, 2013

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INTRODUCTION

Condensed tannins (proanthocyanidins) are oligomers and polymers of the flavan-3-ol monomer with various hydroxylation patterns around both the A and B rings (Figure 1). These flavanoid compounds are commonly found in the wood and bark of plants and exhibit high chemical and biological activities including ultraviolet (UV) absorption, antimicrobial, and antioxidant properties.[1,2] The properties of flavonoids are exploited in numerous established applications, such as leather tanning and wood adhesives,[3] with emerging applications in personal care, nutraceuticals, and pharmaceuticals.[4–7] However, the multiple hydroxyl groups render these materials hydrophilic, an often limiting factor for new applications. An enhancement in lipophilicity would broaden the potential for advancing applications of condensed tannins beyond current uses.

Numerous chemical modifications such as esterification have been reported to improve the lipophilic solubility of condensed tannins. Polyphenolic esterification can be achieved by direct acylation using various reagents including acyl chlorides and anhydrides.[8–12] In the case of the flavanoid catechin, any site selectivity with acyl chlorides requires protection chemistry[13,14] to avoid the alternative of chromatographic separation of the products.[15] An alternative route to prepare flavonoid ester is transesterification. Transesterification is generally reversible and can be catalyzed by a range of compounds including acids, bases, metal oxides, and enzymes such as lipase.[16] The transesterification equilibrium can be forced toward the products by the removal of coproduced alcohol. Therefore, vinyl esters are commonly used as transesterification reagents, as the by-product vinyl alcohol tautomerizes to acetaldehyde preventing reversal.[16–18]

Transesterification of flavonoids and condensed tannins with fatty acid esters is of interest as it may provide different site selectivity compared to conventional acylation approaches. Furthermore, transesterification reagents are often less harmful, cheaper, and more amenable to commercial production than those typically required for acylation. The goal of the reported research was to investigate transesterification as an alternative approach to synthesize lipophilic flavonoid esters. Conditions for transesterification were explored with model compounds, resorcinol and catechin, in combination with various catalyst and solvent systems. Using appropriate conditions, various esters were prepared from both quebracho and radiata pine bark tannins (PBTs) with vinyl acetate (VA) and vinyl laurate (VL). These products were compared to the equivalent esters prepared by conventional acylation using the corresponding acid chlorides.

EXPERIMENTAL

Materials

Lauroyl chloride (LC, Aldrich), VL (Fluka), potassium hydroxide (BDH), triethylamine (TEA, BDH), 4-dimethylaminopyridine

Additional Supporting Information may be found in the online version of this article.

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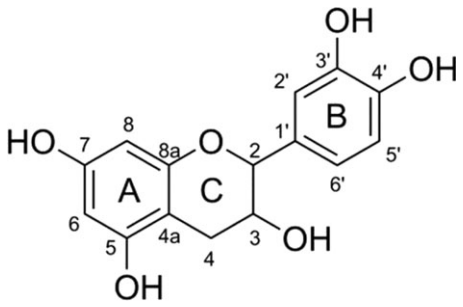

Figure 1. Structure of procyanidin; an example flavan repeat unit common in condensed tannins.

(DMAP, Sigma), hydrochloric acid (Merck), sulfuric acid (Ajax), and toluene-4-sulfonic acid (TSA, BDH) were all of AR grade or better and used as received. (+)-Catechin hydrate (Fluka) was dried under vacuum before use. Resorcinol (BDH) was recrystallized from toluene, and VA (Aldrich) was distilled before use. Quebracho tannin (QT, Colatan GT100) was sourced from Unitan (Argentina). PBT was extracted from the bark of *Pinus radiata* using hot water (90°C), which was subsequently spray-dried. Both tannins were dried under vacuum before use.

Synthesis of Polyphenolic Esters by Transesterification

A general method for the transesterification of polyphenolics with vinyl esters is outlined below, with a summary of the reactions provided in Table I. To a flask the appropriate polyphenol (0.1–2.0 g), solvent (5–25 mL), and catalyst (10% mol/mol) were added. Vinyl ester (two fold excess on the hydroxyl groups) was added, and the reaction was stirred at the specified temperature for 48 h. Further details on the synthesis and work-up of entries **11–15** are provided in the Supporting Information.

Table I. Reaction Conditions and Degree of Substitution (DS) for the Transesterification of Resorcinol, Catechin, Pine Bark Tannin, and Quebracho Tannin with Vinyl Acetate and Vinyl Laurate

Entry	Polyphenol	Reagent	Solvent	Catalyst	T (°C)	DS
1	Resorcinol	VA	Water	KOH	20	0.9[a]
2	Resorcinol	VA	Water	TEA	20	1.0[a]
3	Resorcinol	VA	Water	DMAP	20	1.1[a]
4	Resorcinol	VA	Water	HCl	20	0.0[a]
5	Resorcinol	VA	Water	H_2SO_4	20	0.0[a]
6	Resorcinol	VA	Water	TSA	20	0.0[a]
7	Resorcinol	VA	Water/THF (1 : 1)	KOH	20	0.4[a]
8	Resorcinol	VA	DMSO	TEA	20	0.2[a]
9	Resorcinol	VL	DMSO	TEA	70	0.2[a]
10	Catechin	VA	Water/THF (3 : 1)	KOH	20	0.4[a]
11	Catechin	VL	DMSO	TEA	70	2.0[b]
12	PBT	VA	Water/THF (4 : 1)	KOH	20	0.2[b]
13	QT	VA	Water/THF (4 : 1)	KOH	20	0.3[b]
14	PBT	VL	DMSO	TEA	70	0.9[b]
15	QT	VL	DMSO	TEA	70	4.8[b]

[a]DS of the crude reaction mixture, [b]DS of product mixture after work-up.

Table II. Reaction Conditions and DS of Flavonoid Esters Prepared by Acylation

Entry	Flavonoid	Ratio	DS
16	Catechin	Excess	5.0[a]
17	Catechin	1 : 3	3.4[a]
18	PBT	Excess	8.8[a]
19	PBT	1 : 3	5.5[a]
20	QT	Excess	8.6[a]
21	QT	1 : 3	3.2[a]

[a]DS of product mixture after work-up, before chromatographic separation.

Synthesis of Flavonoid Laurates by Acylation

A general method for the acylation of polyphenolics is outlined below, with a summary of the reactions provided in Table II. Catechin or tannin (0.1–1.0 g) was dispersed in pyridine and/or chloroform (10–15 mL) and heated (75°C) with stirring under a nitrogen blanket. LC (slight excess or 1 : 3 mol ratio) was added drop wise, and the reaction was allowed to proceed for at least 2 h. Further details are provided in the Supporting Information, including the full characterization of catechin pentalaurate.

Characterization

Solution-state NMR spectra were recorded on a Bruker Avance DPX 400 spectrometer with a 5-mm inverse broad band probe (Bruker). Chemical shifts were reported in δ ppm with reference to tetramethylsilane. The degree of substitution (DS) was determined by ^{1}H-NMR spectroscopy using eq. (1) adapted from Luo et al.[12]

$$\mathrm{DS} = \frac{\frac{I_1}{n_1}}{\frac{I_2}{n_2}} \quad (1)$$

where I_1 is the total aliphatic ester integral, n_1 is the number of aliphatic protons of one ester chain, I_2 is the polyphenol unit aromatic integral, and n_2 is the number of aromatic protons based on the hydroxylation pattern of the monomeric polyphenol unit (resorcinol = 4, catechin = 5, PBT = ~ 3.9, and QT = ~ 4.9).[19,20]

Solid-state ^{13}C-NMR spectra were obtained on a Bruker Avance DRX 200 instrument with a 7-mm doubly tuned ^{1}H/X-MAS probe (Bruker). Samples were packed into a zirconia rotor fitted with a Kel-F cap and spun at 5 kHz. A cross polarization-magic angle spinning pulse program was used with a ^{1}H preparation pulse of 5.56 μs, ^{1}H decoupling field of 47 kHz, and an acquisition time of 20 ms.

Infrared spectra were recorded on a Bruker Vector 33 FTIR instrument. Solid samples were prepared as KBr discs and liquids or gels neat between KBr windows.

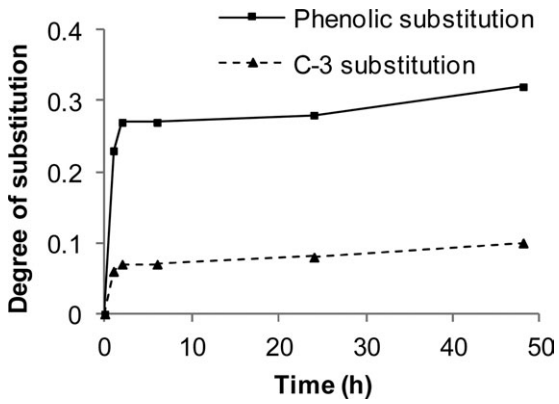

Figure 2. Reaction progress for the base catalysed transacetylation of catechin with vinyl acetate (**10**).

Electrospray ionization-mass spectrometry was performed on a Thermo Finnigan LCQ Deca XP ion trap mass spectrometer. Samples were dissolved in methanol, introduced by direct infusion, and analyzed in negative ion mode with a spray voltage of 4.9 kV.

Thermogravimetric analysis (TGA) experiments were performed using a TA Instruments Q500 thermogravimetric analyzer. Samples were placed in platinum pans and heated at 10°C/min under nitrogen atmosphere.

Differential scanning calorimetry (DSC) experiments were carried out using a TA Instruments Q1000 DSC. Samples were transferred to aluminum pans and run using a heat–cool–heat cycle at a rate of 10°C/min under nitrogen atmosphere.

Antioxidant activity was measured using an ABTS radical scavenging assay as described previously.[21] Experiments were performed using a Varian Cary 300 Bio UV–vis spectrophotometer with triplicate analyses of samples. Results are expressed as micromole trolox equivalent antioxidant capacity (TEAC).

RESULTS AND DISCUSSION

Practicable transesterification conditions were initially established with the phenolic model compound resorcinol and VA using a range of catalysts and solvents (Table I). Conversion to resorcinol acetate in an aqueous system was achieved with a DS greater than or equal to 0.9 with the base catalysts potassium hydroxide, TEA, and DMAP (**1–3**). Hydrochloric, sulfuric, and TSAs proved unsuccessful as catalysts (**4–6**). Reagent miscibility is generally considered as a critical factor to achieve transesterification.[16] This necessitated a suitable solvent system to achieve comiscibility of flavanoids and VL, which inherently have differing solubility. Potential solvent–catalyst combinations included water/tetrahydrofuran with potassium hydroxide (**7**), which gave conversion to resorcinol acetate with a DS of 0.4. A more lipophilic system using dimethyl sulfoxide with TEA (**8**) gave the acetate derivative with a DS of 0.2 at ambient temperatures. Using the latter conditions, resorcinol was transesterified with VL (**9**) to also give a DS of 0.2. However, with a longer pendant chain, gentle heating (70°C) was required to achieve miscibility.

The flavan model compound catechin was acetylated using the general reaction conditions established above for phenolics in water/tetrahydrofuran (**10**). This solvent system, with potassium hydroxide catalyst, solubilized both catechin and VA. Using ^{1}H-NMR spectroscopy, the acetylation of catechin was shown to occur rapidly up to 2 h, after which the rate of reaction was considerably slower (Figure 2). Acetyl substitution occurred at both the secondary alcohol and phenolics as evidenced by downfield shifts of the aromatic, H-2 and H-3 signals. Additionally, phenolic substitution occurred preferentially at the B-ring, with negligible substitution of the A-ring. In contrast, enzyme-catalyzed transesterification generally occurs at the aliphatic hydroxyl groups.[22–25]

Catechin was transesterified with VL, using dimethyl sulfoxide, given its capability to solvate compounds of varying polarity (**11**). Catechin laurate derivatives were fractionated by preparative layer chromatography yielding catechin monolaurate (**11b**), and catechin dilaurate (**11a**) as a minor product. Both products were identified as mixtures of structural isomers. The ^{1}H-NMR spectrum of **11b** revealed this product to be predominantly substituted at the B-ring, as evidenced by downfield shifts of the H-2′, H-5′, and H-6′ signals at ~ 7 ppm (Figure 3). 2D NMR spectroscopy (Figures S1 and S2 of the Supporting Information) was used to identify $^{3}J_{H\text{-}H}$ and $^{4}J_{H\text{-}H}$ couplings of the B-ring allowing unequivocal assignment of signals for both catechin-3′-monolaurate and catechin-4′-monolaurate (Table III). Subsequently, the ratio of C-3′ and C-4′-substituted catechin in sample **11b** was estimated at ~ 1 : 1, indicating no preference for substitution within the catechol B ring. Negligible laurate substitution occurred at the A- or C-rings, confirmed by chemical shifts that were similar to catechin at ~ 6 and 4–5 ppm, respectively. Unsurprisingly, the disubstituted product (**11a**) had laurate groups primarily at the B-ring C-3′ and C-4′ positions, with minimal substitution on either the A or the C rings. The dominant signals in the ^{1}H-NMR spectra (Figure 4) were in accordance with the literature characterization of catechin-3′,4′-dilaurate.[15] Characterization by mass spectrometry identified molecular ions of catechin dilaurate (*m/z* 653 $[M - H]^{-}$) and

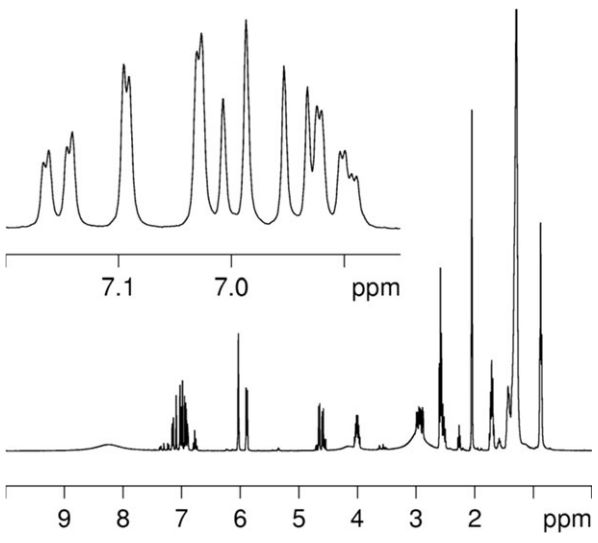

Figure 3. ^{1}H-NMR spectrum of **11b**, with expanded portion showing the B-ring signals for catechin-3′-monolaurate and catechin-4′-monolaurate.

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Table III. Assignment of ^{1}H-NMR Chemical Shifts (σ), Multiplicity (M), and Coupling Constants (J) Identified for Both Catechin-3′-Monolaurate and Catechin-4′-Monolaurate (11b)

Signal	σ (ppm)	*M* (*J* (Hz))
Catechin-3′-monolaurate		
H-6′	7.16	dd (1.8, 8.2)
H-2′	7.09	d (1.8)
H-5′	6.94	d (8.2)
Catechin-4′-monolaurate		
H-6′	6.91	dd (1.7, 8.3)
H-2′	7.03	d (1.6)
H-5′	7.00	d (8.2)

catechin monolaurate (*m/z* 471 $[M - H]^-$) and adducts thereof in samples **11a** and **11b**, respectively. No acetaldehyde condensation products were observed by NMR spectroscopy for **11**, which may arise from the reaction of catechin with the by-produced acetaldehyde.[26] Likewise, the rearrangement of catechin to catechinic acid, known to occur under basic conditions,[27] was not observed by NMR spectroscopy.

To further understand catechin substitution patterns and any preferential substitution positions by laurate groups, partially esterified products were prepared by acylation with lauroyl chloride. Although catechin pentalaurate (**16**) can be prepared with an excess of lauroyl chloride in pyridine, reaction of catechin with 3 mol equivalents of lauroyl chloride (**17**) produced a mixture of substituted products. This mixture was separated by preparative layer chromatography to yield three isomeric mixtures of catechin tetra- (**17a**), tri- (**17b**), and di-laurate (**17c**). Unambiguous assignment of the ^{1}H- and ^{13}C-NMR spectra of catechin pentalaurate (**16**) was aided by 2D NMR spectroscopy, including HMBC and HSQC experiments (Figures S3 and S4 of the Supporting Information). The H-6 signal was confirmed by a $^{4}J_{C-H}$ coupling from C-8a to H-6, with the C-6 and C-8 signals subsequently assigned by $^{1}J_{C-H}$ couplings. A $^{3}J_{C-H}$ coupling between

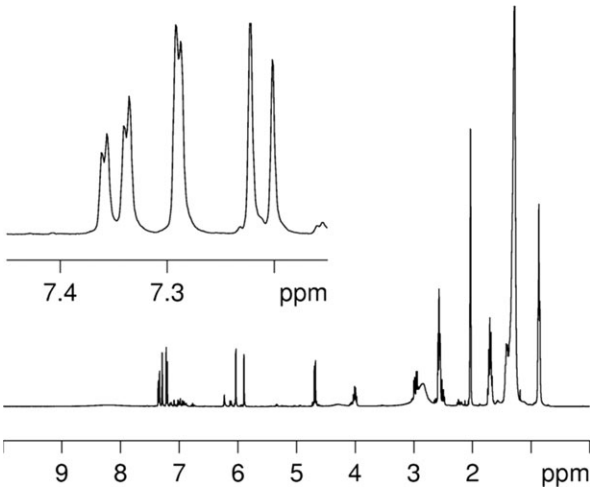

Figure 4. ^{1}H-NMR spectrum of catechin dilaurate (**11a**) with expanded portion showing the B-ring signals.

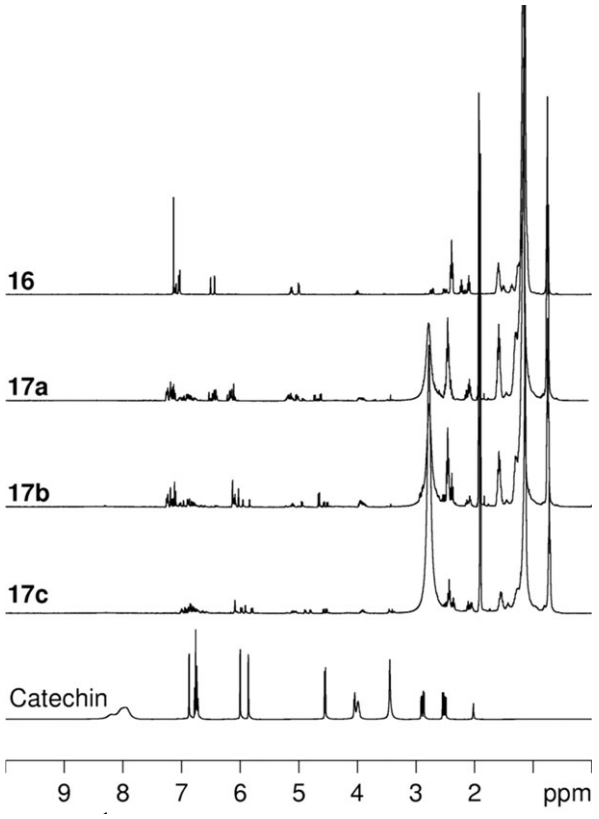

Figure 5. ^{1}H-NMR spectra of catechin penta-, tetra-, tri-, and di-laurate (**16**, **17a**, **17b**, and **17c**) compared to unmodified catechin.

C-5 and H-4 confirmed that the C-5 signal was of lower ppm shift than C-7. Furthermore, the assignment of C-2′, C-5′, and C-6′ was confirmed by $^{1}J_{C-H}$ couplings. In the case of the isomeric tetralaurate product **17a**, NMR revealed downfield shifts of A-, B-, and C-ring protons, indicating that acylation occurred at all hydroxyl groups (Figure 5). In contrast, the tri- and di-laurates (**17b** and **17c**) show less obvious downfield shifts of the A-ring protons. As observed for transesterification, this indicates that initial acylation also occurs preferentially at the B-ring phenols. This is in agreement with Jin and Yoshioka[15] who similarly reacted catechin with 2 mol equivalents of lauroyl chloride and isolated catechin-3′,4′-dilaurate and catechin-3,3′,4′-trilaurate. The dissociation constants for catechin show that ionization occurs first at the B-ring hydroxyl groups,[28] further supporting an initial preference for the esterification of the B-ring.

Using reaction chemistry established with catechin, two condensed tannins were reacted using both transesterification and direct acylation approaches. In the case of transesterification, both the pine bark and QT were reacted with VA and VL. The transacetylation of both tannins yielded a brown solid that was easily isolated (**12** and **13**). Integration of the ^{1}H-NMR spectra was used to estimate the DS (Table I), where PBT and QT have ~ 5.2 and 4.3 hydroxyl groups per flavan unit, respectively.[19,20] The DS was relatively low, less than 1, with a greater DS achieved for QT (**13**) compared to PBT (**12**).

For the transesterification of PBT with VL, the work-up proved simple with the excess VL removed by decanting and the product

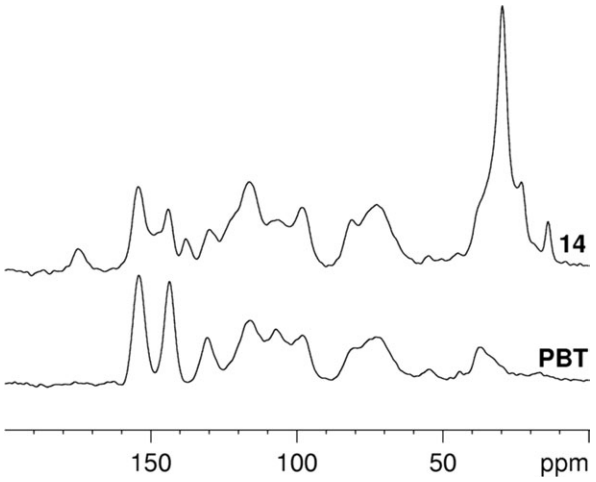

Figure 6. ^{13}C CP–MAS NMR spectra of pine bark tannin and the transesterified laurate derivative (**14**).

precipitated to yield a brown solid (**14**). In contrast, the QT product (**15**) proved more difficult to isolate, requiring additional work-up by preparative layer chromatography to isolate the products. Again, the DS for PBT (**14**) was considerably less than that of QT (**15**). This likely reflects the work-up procedure required for the QT product, with only a highly substituted portion of the crude product being isolated upon chromatography. Solid-state ^{13}C-NMR spectroscopy of the PBT laurate (**14**) showed the C-3′ and C-4′ signal at 144 ppm split and moved upfield, while the C-5, C-7, and C-8a signal of the A-ring at 154 ppm showed no change (Figure 6). This confirmed that most substitution was occurring selectively at the B-ring, as similarly observed for catechin.

Both PBT and QT were reacted with excess and 3 mol equivalents of lauroyl chloride to achieve variable levels of laurate substitution (Table II). The high-hydrolytic reactivity of the acid chloride liberated lauric acid when mixed with the hydroscopic tannins. This free acid resulted in strong emulsions during work-up and proved difficult to remove. Chromatographic separation was required to isolate a product with minimal lauric acid. These observations provide some practical context to potential difficulties in preparing the tannin laurates by this route. Both samples **18** and **20** had a DS greater than the total number of hydroxyl groups present in the starting materials. This higher DS may be attributed to the presence of some residual lauric acid in the products as well as acylation of other components present within the tannin extracts such as carbohydrates.

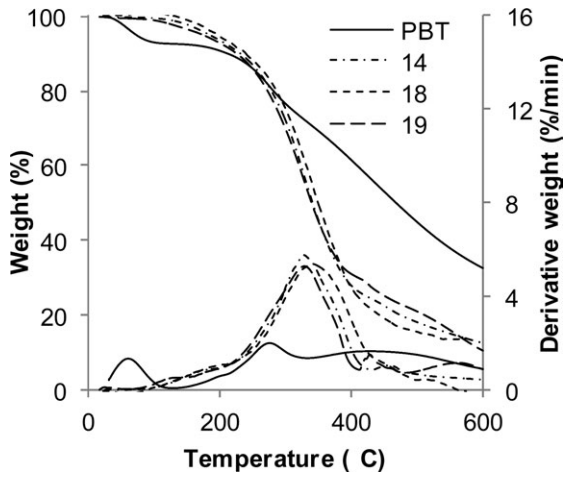

Figure 7. TGA thermogram of pine bark tannin laurates prepared by transesterification (**14**) and direct acylation (**18** and **19**).

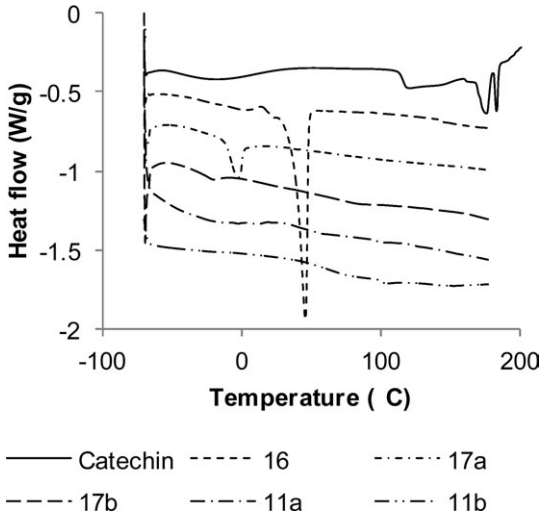

Figure 8. DSC thermogram (second heating cycle) of catechin laurates prepared by direct acylation (**16**, **17a**, and **17b**) and transesterification (**11a** and **11b**) compared with catechin.

The flavonoid laurate products were characterized both thermally and for their antioxidant properties. With TGA, both pine bark and QTs exhibit a notable weight loss below 100°C, attributable to water loss (Figure 7).[29] Upon further heating, the tannins show initial degradation above 150°C with residual weights of ~ 40% at 600°C. The tannin laurates did not show any water loss, indicating their inherent hydrophobicity. The modified PBTs (**14**, **18**, and **19**) begin to degrade at temperatures greater than 100°C, with rapid weight loss occurring above 200°C to give residual weights of ~ 10% at 600°C. The weight loss observed between 200 and 400°C was predominately associated with laurate group loss, typical of tannin esters.[12,29] In contrast, the QT laurates (**20** and **21**) were more stable compared to the PBT laurates, with the onset of initial degradation occurring above 200°C.

DSC was used to investigate any thermal transitions or melt behaviors. In the case of catechin laurate esters, an endothermic absorption was observed between ~ −20 and 45°C, associated with melting behavior (Figure 8). The melt transition of catechin pentalaurate (**16**) was relatively sharp in comparison with the broader endothermic features of the tetra- and tri-substituted catechin samples. Moreover, this melt feature occurred at lower temperature, becoming broader with lower levels of substitution. A broadened melt feature is consistent with the products being a mixture of isomers, as similarly reported for other flavonoid derivatives.[12] The PBT laurates showed similar behavior to the catechin derivatives. An endothermic transition

was observed slightly above room temperature (48°C) for samples **18** and **19**. This melt feature was not evident for the transesterified derivative (**14**) with low DS, which was also consistent with the findings of catechin. In contrast, the QT laurates showed negligible thermal features on heating.

The antioxidant capacity of PBT decreased upon esterification. In comparison with unmodified PBT, the laurate derivative (**14**) had reduced antioxidant activity from 5260 to 2430 TEAC. This was consistent with Jin and Yoshioka[15] who found that partial esterification of catechin, especially at the B-ring, reduced the radical scavenging activity. However, if applied to a lipophilic matrix, the enhancement of lipophilicity would likely compensate for the decrease in antioxidant capacity.[15]

CONCLUSION

Transesterification has been demonstrated as a viable alternative to acylation for esterifying flavanoids, with both short and long-chain vinyl esters. The generality observed suggests that vinyl esters of varying chain length could be used if a homogenous reaction mixture is retained. Transesterification achieved partial substitution that was generally limited to the formation of mono- and di-esters. The substitution patterns for transesterification were found to be similar to those for acylation, with initial esterification occurring preferentially at the flavonoid B-ring. Transesterification offered the benefits of using more benign reagents, with subsequent advantages regarding safety, expense, and reduced by-products. The absence of hydrolysis reactions that form free fatty acids greatly simplified the work-up procedure. The isolated flavan laurate products were lipophilic, thermally stable to at least 100°C, and determined to soften and melt near room temperature. In addition, partially substituted PBT laurate retained moderate antioxidant capacity, offering promise for therapeutic applications or as an additive in polymer system.

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