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The potential and practical applications of acylated flavonoids

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Flavonoids have many beneficial health effects. However, their practical applications are often strongly limited due to low solubility and stability in lipophilic media. In order to eliminate this drawback, enzymatic acylation with fatty acids has been introduced. Besides altering physico-chemical properties, bioavailability and biological properties of maternal compounds may also be improved. Although the research dealing with flavonoid ester synthesis is a matter of few years, and several aspects need to be clarified, there is a promising prospect of future application of these selectively acylated derivatives in therapy, cosmetics, and dietetic fields.

1. Introduction

Flavonoids comprise a widely distributed group of polyphenolic plant secondary metabolites. Perhaps the most active area of flavonoid research is related to their possible bio medicinal contribution to human health (Harborne and Williams 2000). An inverse correlation between the intake of certain polyphenols and the risk of cardiovascular disease, cancer and other age related diseases has been observed in epidemiological studies (Li et al. 2007; Naruszewicz et al. 2007; Walle 2007; Heo and Lee 2005; Schijlen et al. 2004). The protective effect of flavonoids is mainly attributed to their free radical scavenging and antioxidant activity (Lin and Weng 2006; Rice-Evans et al. 1996; Frankel et al. 1993), but they also possess other physiological properties which are beneficial to human health such as antimicrobial (Proestos et al. 2006; Martini et al. 2004), antiviral (Shimura et al. 1999; Wang et al. 1998), anticarcinogenic (Linsalata and Russo 2008; Brownson et al. 2002; Depeint et al. 2002; Middleton et al. 2000; Wang et al. 1998), anti-inflammatory (Narayana et al. 2001; Kim et al. 1998), anti-allergic (Yamamura et al. 1998), vasodilatory (Zenebe and Pechanova 2002), and enzyme-inhibitory effects (Vasquez-Martinez et al. 2007; Dangles and Dufour 2006; Middleton et al. 2000; Robert et al. 1990).

For the numerous health-beneficial activities of flavonoids, there is a growing interest in their use in protection or treatment of various disorders. However, the application of these compounds is limited due to solubility and stability problems during processing and storage. The development of anthocyanin colorants has led to discovery of stable anthocyanin molecules with complex patterns of glycosylation and acylation that exhibit remarkable stability to pH changes, heat treatment, light exposure (Guisti and Wrolstad 2003) and bioavailability (Moussou et al. 2007).

These selectively modified natural compounds are promising targets for industrial purposes due improved physicochemical and biological properties in different fields. Currently, several formulations containing these novel compounds with modified properties have been patented. In this review paper, we provide a brief overview of the potential and significance of the flavonoid acylation and important inventions in application fields patented up-to-date.

2. Flavonoid acylation in plants

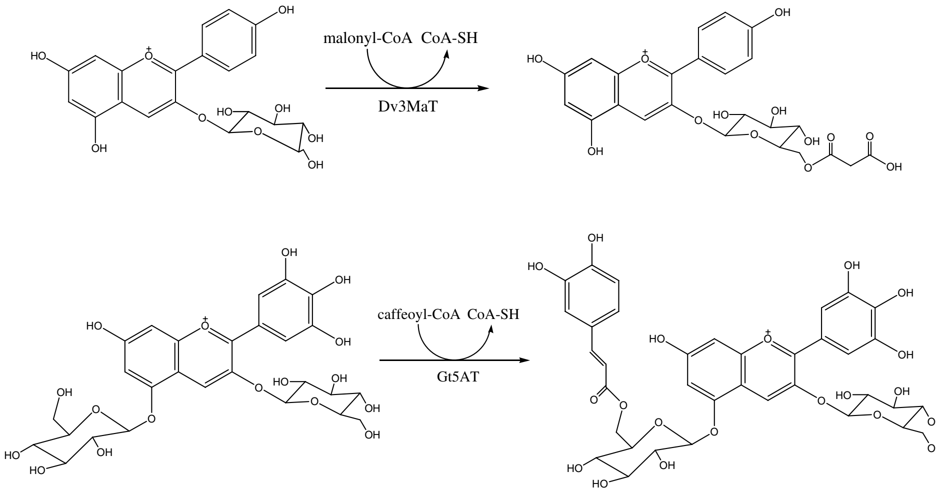
2.1. Biosynthesis

Flavonoid biosynthesis is one of the best studied and characterized among the secondary metabolic pathways. These

Table: Acylating agents found in flavonols, flavones (Williams 2006) and anthocyanins* (Andersen and Jordheim 2006)

Aliphatic acids	Aromatic acids
Acetic*	Benzoic
Malonic*	p-Hydroxybenzoic*
Lactic	Gallic*
Vinylpropionic	Cinnamic
Succinic*	p-Coumaric*
Butyric	Caffeic*
Isobutyric	Ferulic*
3-Methylbutyric	Isoferulic
Crotonic	Sinapic*
n-Butanoic	Methylsinapic
Isovaleric	
Quinic	
Tiglic	
Malic*	
Tartaric*	

Scheme



Regiospecific acyl transfer reactions catalyzed by some AATs (Davies and Schwinn 2006).
Enzymes: malonyl-CoA : anthocyanidin 3-*O*-glucoside-6″-*O*-malonyltransferase (Dv3MaT), hydroxycinnamoyl-CoA : anthocyanin 5-*O*-glucoside 6‴-*O*-hydroxycinnamoyltransferase (Gt5AT)

compounds are derived from phenylalanine and malonyl-coenzyme A. Most enzymes involved in this pathway and their corresponding genes have been characterized (Davies and Schwinn 2006).

The huge diversity in flavonoid structures is due to modifications of the basic skeleton by specific reactions, including glycosylation, methylation and acylation (Schijlen et al. 2004). In some plant species, the last step in the flavonoid biosynthesis is terminated with acylation. This reaction is catalyzed by various acyltransferases which are responsible for the transfer of either aromatic or aliphatic acyl groups from a CoA-donor molecule to hydroxyl residues of flavonoid sugar moieties (Davies and Schwinn 2006). Some of the aromatic and aliphatic acids found in flavone, flavonol and anthocyanin class are listed in the Table (Andersen and Jordheim 2006; Williams 2006).

Many flavonoids undergo acylation most commonly with malonic, coumaric, caffeic or ferulic acid (Bloor and Abrahams 2002; Fujiwara et al. 1998). Acylation is widespread especially among anthocyanins; more than 65% are reported to be acylated (Andersen and Jordheim 2006). The acylation of anthocyanins is catalyzed by anthocyanin acyltransferases (AATs) and takes place after anthocyanidin 3-*O*-glucoside formation. Some examples of acylations carried out by these enzymes are shown in the Scheme.

AATs can be classified into two distinct categories on the basis of their acyl-donor specificity, i.e. aliphatic acyltransferases and aromatic acyltransferases. Aliphatic acyltransferases do not act on aromatic acyl-CoAs, such as *p*-coumaroyl-CoA and caffeoyl-CoA, and vice versa. Many AATs have been shown to have strict specificities for the position of the acylation of anthocyanins. Depending on the plant species, AATs are known to catalyze 6″-*O*- and 4‴-*O*- and 6‴-*O*-acylation (Nakayama et al. 2003).

2.2. *Effects of acylated flavonoids*

While the exact role of plant acylation is not yet fully understood, it is known that these modifications modulate the physiological activity of the resulting flavonoid by altering solubility, stability, reactivity and interaction with cellular targets (Ferrer et al. 2008).

It has been recognized that the flavonoids acylated with certain aromatic carboxylic acids improved thermostability and light-resistivity of certain flavonoids and thus are involved in physiological actions such as UV-absorbing capacity and the radical scavenging ability (Delazar et al. 2005; Ishihara and Nakajima 2003; Alluis and Dangles 1999; Jungblut et al. 1995). This reaction (mainly with *p*-coumaric acid) contributes to the pigment stabilization of flavonoids (especially anthocyanins) by aggregation or stacking due to the inter- and intramolecular hydrophobic interactions or the π-π stacking between the flavonoid skeleton and the aromatic ring in the acyl moiety (Ishihara and Nakajima 2003), which protects the oxonium ion from decomposition without the need for any co-pigment or metal cation (Cevallos-Casals and Cisneros-Zevallos 2004; Ishihara and Nakajima 2003; Figueiredo et al. 1999). That's why flavone or flavonol glycosides having hydroxcinnamyl acylation linked through sugars are marked as being UV-protective (Harborne and Williams 2000).

The aliphatic acylation of anthocyanins (malonylation) is important for enhancing the pigment solubility in water, protecting glycosides from enzymatic degradation and stabilizing anthocyanin structures (Nakayama et al. 2003). Several *in vitro* observations suggest that acylation with malonic acid or sinapic acid is crucial for efficient flavonoid accumulation. Acylation might be a prerequisite molecular tag for efficient vacuolar uptake of flavonoids (Kitamura 2006; Nakayama et al. 2003; Boller and Wiemken 1986).

Some acylated flavonoids have been found to be involved in plant-insect interactions; they act as phytoalexins, oviposition stimulants, pollinator attractants (Iwashina 2003), and insect antifeedants (Harborne and Williams 1998). With respect to novel biological activities, acylation of flavonoids can result in changes in pigmentation (Bloor 2001), insect antifeedant activity (Harborne and Williams 1998) and antioxidant properties (Alluis and Dangles 1999). Anthocyanin acylation should be of nutritional and biomedical importance because some of the bioactivities of anthocyanins have been shown to be strongly modulated by acylation (Nakayama et al. 2003).

3. *In vitro* synthesis of acylated flavonoids

Flavonoid acylation, as has been described, provides a useful tool for flavonoid ester formation in plants with improved characteristics. Therefore, scientists have tried to take an advantage of this naturally occurring phenomenon and implement acylation methods into laboratories. However, the use of acyltransferases as modifying agents is rather inconvenient, as they require corresponding acyl-coenzyme A, which must be either used in stoichiometric amounts or regenerated *in situ*. To solve this problem, the chemical approach was first investigated, which possessed a low degree of regioselectivity of the esterification and drastic operating conditions had to be used (Patti et al. 2000). Later on, hydrolytic enzymes (lipases, esterases and proteases) have been recognized as a useful substitute due to their large availability, low cost, chemo-, regio- and enantioselectivity, mild condition processing and no need of cofactors (Collins and Kennedy 1999; Nagasawa and Yamada 1995).

Presently, the lipase-catalyzed flavonoid esterification in organic media is a well-studied and mastered technique for synthesis of selectively modified flavonoids. The results suggest that a high degree of conversion to desired esters can be achieved when optimal working conditions are used. The key factors, which influence regioselectivity and the performance of the enzymatic acylation of flavonoids, include type and concentration of enzyme, the structure and concentration of the substrates (acyl donor, acyl acceptor and their ratio), the nature of the reaction media, water content in the media, reaction temperature and the nature of reaction (reviewed in Chebil et al. 2007; Chebil et al. 2006).

4. Biological properties and practical significance of esterified flavonoids

It has been proposed that selectively acylated flavonoids with different acyl donors may not only improve physicochemical properties of these molecules (Ishihara et al. 2003), but also introduce various beneficial properties to the maternal compound such as penetration through the cell membrane (Suda et al. 2002; Kodelia et al. 1994) and improve existing or confer novel bioactivities, including antioxidant (Katsoura et al. 2006; Mellou et al. 2005), antimicrobial (Mellou et al. 2005), anti-proliferative (Mellou et al. 2006) and cytogenetic (Kodelia et al. 1994) properties.

Since the enzymatic preparation of flavonoid derivatives is a matter of few years, the practical applications have just been emerging. There are several patented inventions available to date, which are oriented on the flavonoid ester production and their use for the manufacture of pharmaceutical, dermopharmaceutical, cosmetic, nutritional or agri-foodstuff compositions (Fukami et al. 2007; Moussou et al. 2007; Ghoul et al. 2006; Moussou et al. 2004; Bok et al. 2001; Perrier et al. 2001; Otto et al. 2001; Nicolosi et al. 1999; Sakai et al. 1994).

4.1. Pharmaceuticals

In recent years, coronary artery diseases, such as atherosclerosis and hypercholesterolemia, a major cause of deaths. Novel acylated flavanone derivatives are effective in treating or preventing elevated blood lipid level-related diseases, e.g., hyperlipidemia, arteriosclerosis, angina pectoris, stroke and hepatic diseases since they exert inhibitory effects on the acylcolicholesterol acyl transferase activity; and the HMG-CoA reductase activity. Further, in spite of their potent efficacies, the flavanone derivatives exhibit no toxicity or mitogenicity in tests using mice (Bok et al. 2001).

The 6″-*O*-esterification of kaempferol-3-*O*-glucoside (astragalin) with *p*-coumaric acid was found to increase anti-inflammatory activity eight times compared to the initial flavonoid, while addition of another *p*-coumaroyl group at 2″ position gave an activity 30 times greater than that of astragalin (Harborne and Williams 2000). Another kaempferol derivative, kaempferol 3-(2″,3″-di-*E*-*p*-coumaroylrhamnoside), was found to possess a cytotoxic effect because it significantly modulated the proliferation of HL60 (a promyelocytic cell line) and MOLT3 (a T-ALL with phenotypic characteristics of cortical thymocytes) (Mitrokotsa et al. 1993). Also Demetzos et al. (1997) synthesized novel flavonoid esters with cytotoxic activity. These acetylated esters of tiliroside exhibited strong cytotoxic effect against four leukemic cell lines (HL60, DAUDI, HUT78 and MOLT3), whilst the maternal compound had no effect (Demetzos et al. 1997). Tricin-7-*O*-β-(6″-methoxycinnamic)-glucoside, a flavone from sugarcane, was found to exhibit *in vitro* antiproliferative activity against several human cancer cell lines, with higher selectivity toward cells of the breast resistant NIC/ADR line (Duarte-Almeida et al. 2007).

Mellou et al. (2006) provided the evidence that flavonoid derivatives esterified with polyunsaturated fatty acids were able to decrease the production of vascular endothelial growth factor by K562 human leukemia cells unlike the initial flavonoids, indicating that these novel compounds might possess improved anti-angiogenic and anti-tumor properties. In a previous work Mellou et al. (2005) carried out the enzymatic acylation on Greek endemic plants and reported that this modification increased both their antioxidant activity towards isolated low-density lipoproteins (LDL) and serum model and antimicrobial activity against two Gram-positive bacteria the *Staphylococcus aureus* and *Bacillus cereus*. Also, Katsoura et al. (2006) found out that biocatalytic acylation of rutin with various acyl donors affects its antioxidant potential towards both isolated LDL and total serum model *in vitro*. A significant increase in antioxidant activity was observed for rutin-4‴-oleate. Parejo et al. (2005) examined quercetagetin glycosides acylated with caffeic and *p*-coumaric acid for antioxidant activity. They found out that these compounds exhibited a high radical scavenging activity in comparison with reference compounds.

Fatty acid ester derivatives of catechins are described as having antitumorigenesis promoting activity or 5-α reductase inhibiting activity, as well as antibacterial activity (Fukami et al. 2007). Since these acylated catechin compounds have a much more superior solubility in fats and oils than any catechins previously known, they may be used as a highly effective antioxidative agents (Sakai et al. 1994). A different catechin derivative, 3-*O*-octanoyl-(+)-catechin, was synthesized by Aoshima et al. (2005) by incorporation of an octanoyl chain into (+)-catechin. This ester was found to inhibit the response of ionotropic gamma-aminobutyric acid receptors and Na+/glucose cotransporters expressed in *Xenopus* oocytes in a noncompetitive manner, more efficiently than catechin. Moreover, it induced a nonspecific membrane current and decreased the membrane potential of the oocyte. This newly synthesized catechin derivative possibly binds to the lipid membrane more strongly than do catechin, (−)-epicatechin gallate, or (−)-epigallocatechin-3-gallate and as a result perturbs the membrane structure (Aoshima et al. 2005).

Kodelia et al. (1994) investigated cytological properties of enzymatically prepared ester of rutin and trichloroethylbutyrate. They found out that this rutin-ester caused significantly higher levels of micronuclei than rutin alone what can be considered as a manifestation of a higher action of the agent on the chromosome owing to its easier penetration in to the cell after its esterification (Kodelia et al. 1994).

4.2. *Food*

The major contribution of acylated flavonoids in food industry lies in stability and solubility improvement of initial molecules for instance by reducing oxidation in oil/fat-based food, desirable modification of unwanted sensory properties of certain flavonoids, taking advantage of pigment stabilization by the means of flavonoid acylation, or other food characteristics. Furthermore, selectively acylated flavonoids may cause significant changes in their bioavailability and bioactivity and thus may play a role in preventing diseases when consumed.

Flavonoid acylation is a useful tool for modification of sensory properties of food. While flavonoids provide a variety of health benefits, flavonoid-containing food often suffers from bitter and astringent taste. Degenhardt et al. (2007) found that certain glycosylation and acylation patterns can effectively modulate these negative taste factors in edible preparations, pharmaceutical preparations and cosmetics with mouth contact (i.e. tooth paste, mouth wash). Both the taste intensity and the taste profile perception are improved by the novel compounds.

Ghoul et al. (2006) introduced a process for the selective preparation of acylated flavonoid glycosides with improved stability and solubility in various preparations antioxidative effect remaining intact or being improved. Another particular advantage obtained by these modified flavonoids is that bifunctional molecules with higher biological activity were formed.

Free unsaturated fatty acids represent a potential risk because they are highly reactive and by creating free radicals cause undesirable damage in food. Enzymatic synthesis of flavonoids with unsaturated fatty acids has been found to be a useful solution for the stabilization of these highly oxidizable acids (Mellou et al. 2006).

Another important benefit lies in the use of acylated anthocyanins as food colorants which can serve as a useful alternative to synthetic additives (Giusti and Wrolstad 2003; Fox 2000; Asen et al. 1979). Discovering of acylated anthocyanins with increased stability have shown that these pigments may provide food products with desirable color and stability at wide pH range. Examples of suitable acylated anthocyanin sources may be radishes, red potatoes, red cabbage, black carrots, and purple sweet potatoes (reviewed in Giusti and Wrolstad 2003). The invention of Asen et al. (1979) refers to a stable food colorant from a natural source. It relates to an anthocyanin isolated from the Heavenly Blue morning glory (*Ipomoea tricolor* Cav cv), peonidin 3-(dicaffeylsophoroside)-5-glucoside, which is characterized by the stability of colors ranging from purplish-red to blue produced in food and beverage products at pH values from about 2.0 to about 8.0. Fox (2000) have reported the invention referring to a stable, ruby red natural colorant (anthocyanins acylated with chlorogenic acid) derived from purple sunflower hulls which is useful as a coloring agent in food products, cosmetics, pharmaceuticals and other materials.

4.3. *Cosmetics*

The majority of cosmetic or dermopharmaceutical compositions consist of a fatty phase, the oily products of which have a certain tendency to oxidize, even at room temperature. The consequence of this oxidation is to profoundly modify the properties, which makes them unusable after a variable time period. In order to protect the compositions with respect to these oxidation phenomena, it is common practice to incorporate protective agents which act as antioxidizing agents (N'guyen 1995).

By virtue of the skin-protecting and skin-cleansing properties of flavonoids and their effects against ageing, against skin discoloration and on the appearance of the skin, they have been used as constituents of cosmetic or dermopharmaceutical compositions. They also act on the mechanical properties of the hair (Ghoul et al. 2006).

Moussou et al. (2007) found that the esters of flavonoids with omega-substituted C6 to C22 fatty acids have the property to protect the skin cells against damages caused by UV radiation. These esters of flavonoids according to the invention protect skin cells against UVA and UVB radiation in a more effective manner than the flavonoids alone. Moreover, these esters demonstrated their property to stimulate the glutathione metabolism of human skin cells after UVA irradiation, i.e., to stimulate their cellular defenses. They have also anti-inflammatory and soothing properties, as demonstrated by the inhibition of released protein kinase PGE2 after UVB irradiation. Thereby these flavonoid esters may be used to protect the skin and scalp and/or to fight against UV and sun damage, erythema, sunburn, mitochondrial or nuclear DNA damage, to prevent or fight photo-aging, providing improvement for signs of ageing as skin wrinkles, elasticity is lost and a decrease in skin thickness (Moussou et al. 2007).

Perrier et al. (2001) discovered that specific flavonoid esters can be stabilized while preserving their initial properties, particularly of free radical inhibition and enzyme inhibition, and for applications associated with these properties: venous tonics, agents for increasing the strength of the blood capillaries, inhibitors of blotchiness, inhibitors of chemical, physical or actinic erythema, agents for treating sensitive skin, decongestants, draining agents, slimming agents, anti-wrinkle agents, stimulators of the synthesis of the components of the extracellular matrix, toners for making the skin more elastic, and anti-ageing agents (Perrier et al. 2001).

5. Conclusion

Acylation is a powerful tool for the structural and functional modification of flavonoids. Flavonoid acylation may improve physico-chemical and biological properties of these substances. There has been a growing interest in the flavonoid biotransformation by specific enzyme-mediated acylations. It has been established that regioselective modification of the flavonoid skeleton may increase certain biological activities or confer novel properties. This approach also enables the use of flavonoid fatty acid esters in oil-based systems. The lipophilization of flavonoids also causes greater affinity to the cell membrane and may improve their bioavailability. Thus, these acylated derivatives are promising candidates to be used in cosmetic, pharmaceutical and nutritional preparations for preventive and/or therapeutical purposes.

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