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Effects of synthetic alkamides on Arabidopsis fatty acid amide hydrolase activity and plant development

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ABSTRACT

Alkamides and *N*-acylethanolamines (NAEs) are bioactive, amide-linked lipids that influence plant development. Alkamides are restricted to several families of higher plants and some fungi, whereas NAEs are widespread signaling molecules in both plants and animals. Fatty acid amide hydrolase (FAAH) has been described as a key contributor to NAE hydrolysis; however, no enzyme has been associated with alkamide degradation in plants. Herein reported is synthesis of 12 compounds structurally similar to a naturally occurring alkamide (*N*-isobutyl-(2*E*,6*Z*,8*E*)decatrienamide or affinin) with different acyl compositions more similar to plant NAEs and various amino alkyl head groups. These "hybrid" synthetic alkamides were tested for activity toward recombinant Arabidopsis FAAH and for their effects on plant development (*i.e.*, cotyledon expansion and primary root length). A substantial increase in FAAH activity was discovered toward NAEs *in vitro* in the presence of some of these synthetic alkamides, such as *N*-ethyllauroylamide (**4**). This "enhancement" effect was found to be due, at least in part, to relief from product inhibition of FAAH by ethanolamine, and not due to an alteration in the oligomerization state of the FAAH enzyme. For several of these alkamides, an inhibition of seedling growth was observed with greater results in FAAH knockouts and less in FAAH over-expressing plants, suggesting that these alkamides could be hydrolyzed by FAAH *in planta*. The tight regulation of NAE levels *in vivo* appears to be important for proper seedling establishment, and as such, some of these synthetic alkamides may be useful pharmacological tools to manipulate the effects of NAEs *in situ*.

1. Introduction

Alkamides and *N*-acylethanolamines (NAEs) are small, amide-containing lipids (Chapman et al., 1998; López-Bucio et al., 2006). The compositions of alkamides and NAEs vary in chain length and the number of double bonds in their acyl groups (Boonen et al., 2012; Chapman, 2004; Chapman et al., 1998, 1999). The structural diversity of alkamides is greater than NAEs due to the composition of the amino alkyl head group, which can include but are not limited to butyl, isopropyl, or isobutyl moieties (Boonen et al., 2012; López-Bucio et al., 2006).

NAEs occur widely and are used as signaling substances in animals, plants and some microorganisms (Blancaflor et al., 2014; Coulon et al., 2012; Okamoto et al., 2007). In plants, *N*-palmitoylethanolamine (NAE 16:0), *N*-oleoylethanolamine (NAE 18:1), *N*-linoleoylethanolamine (NAE 18:2) and *N*-linolenoylethanolamine (NAE 18:3) are generally the most abundant NAE types (Blancaflor et al., 2014; Chapman et al., 1999) reflecting both the common fatty acids found in membranes and their biosynthetic origin. Alkamides, on the other hand, appear to be more restricted in their distribution; they have been found in about 10 plant families and some fungi (Boonen et al., 2012; López-Bucio et al., 2006; Ramírez-Chávez et al., 2004) but have not been reported in animal systems. While NAEs are derived from membrane phospholipid precursors, alkamides in plants are likely made from amino acid

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precursor (Cortez-Espinosa et al., 2011). Despite these structural and biosynthetic differences, NAEs and alkamides exhibit some similar biological effects when applied to plants suggesting there may be some overlap in metabolism and/or targets of both groups of compounds.

Different alkamide moieties have been reported in plants (Boonen et al., 2012; López-Bucio et al., 2006; Ramírez-Chávez et al., 2004; Hajdu et al., 2014). The most common naturally-occurring alkamide has an acyl group containing a 2*E* double bond and an isobutyl head group, identified as *N*-isobutyl-(2*E*,6*Z*,8*E*)decatrienamide, also called affinin (Ramírez-Chávez et al., 2004). Different plants such as *Echinacea purpurea* or *Echinacea angustifolia* can accumulate high levels of alkamides in certain organs, and extracts have been used for many years for their purported therapeutic benefits (López-Bucio et al., 2006). Recently several alkamide compounds such as *N*-benzyl-(9*Z*,12*Z*)octadecadienamide were shown to inhibit FAAH activity (IC_{50} = 4 μM) and/or to interfere with the cellular uptake of *N*-(2-hydroxyethyl)-(5*Z*,8*Z*,11*Z*,14*Z*)eicosatetraenamide (also named anandamide) the most widely recognized NAE in the mammalian endocannabinoid system (Hajdu et al., 2014).

NAEs have been associated with several functions in plants such as seedling establishment (Blancaflor et al., 2003; Chapman, 2004; Chapman et al., 1999; Kilaru et al., 2007; Wang et al., 2006), flowering (Teaster et al., 2012), responses to pathogens (Kang et al., 2008; Tripathy et al., 1999), inhibition of phospholipase (Austin-Brown and Chapman, 2002) and lipoxygenase (Keereetaweep et al., 2010). *N*-(2-Hydroxyethyl)dodecanamide (also referred to as *N*-lauroylethanolamine or NAE 12:0) has been widely used to study the effects of NAEs especially on plant root development (Chapman, 2004; Coulon et al., 2012). Although the precise mechanism remains unknown, NAEs, in part, appear to interact with the abscisic acid signaling pathway to mediate their negative effects on seedling growth (Keereetaweep et al., 2013; Teaster et al., 2007).

Besides their medicinal properties, alkamides are also known for their insecticidal effects (Ramírez-Chávez et al., 2004). Recently, a new function for alkamides has been ascribed to affinin, and a reduced form of this lipid (*N*-isobutyl-(2*E*)decanamide and *N*-isobutyldecanamide), for the modulation of Arabidopsis seedling growth (Ramírez-Chávez et al., 2004). Application of exogenous affinin at levels below 28 μM increased the primary root length, whereas affinin concentrations above 28 μM inhibited root development (Ramírez-Chávez et al., 2004). The effects of alkamides on plant development may operate through the cytokinin-signaling pathway to control the activity of the plant meristem and differentiation processes (López-Bucio et al., 2007). In addition, genetic evidence suggests that alkamides modulate lateral root formation via interaction with the jasmonic acid signaling pathway (Méndez-Bravo et al., 2011).

Thus, NAEs and alkamides share some similarities and differences in structure, distribution and their influence on plant development (López-Bucio et al., 2006, 2007). The studies of the effects of *N*-decanoylethanolamide (NAE 10:0) on root development

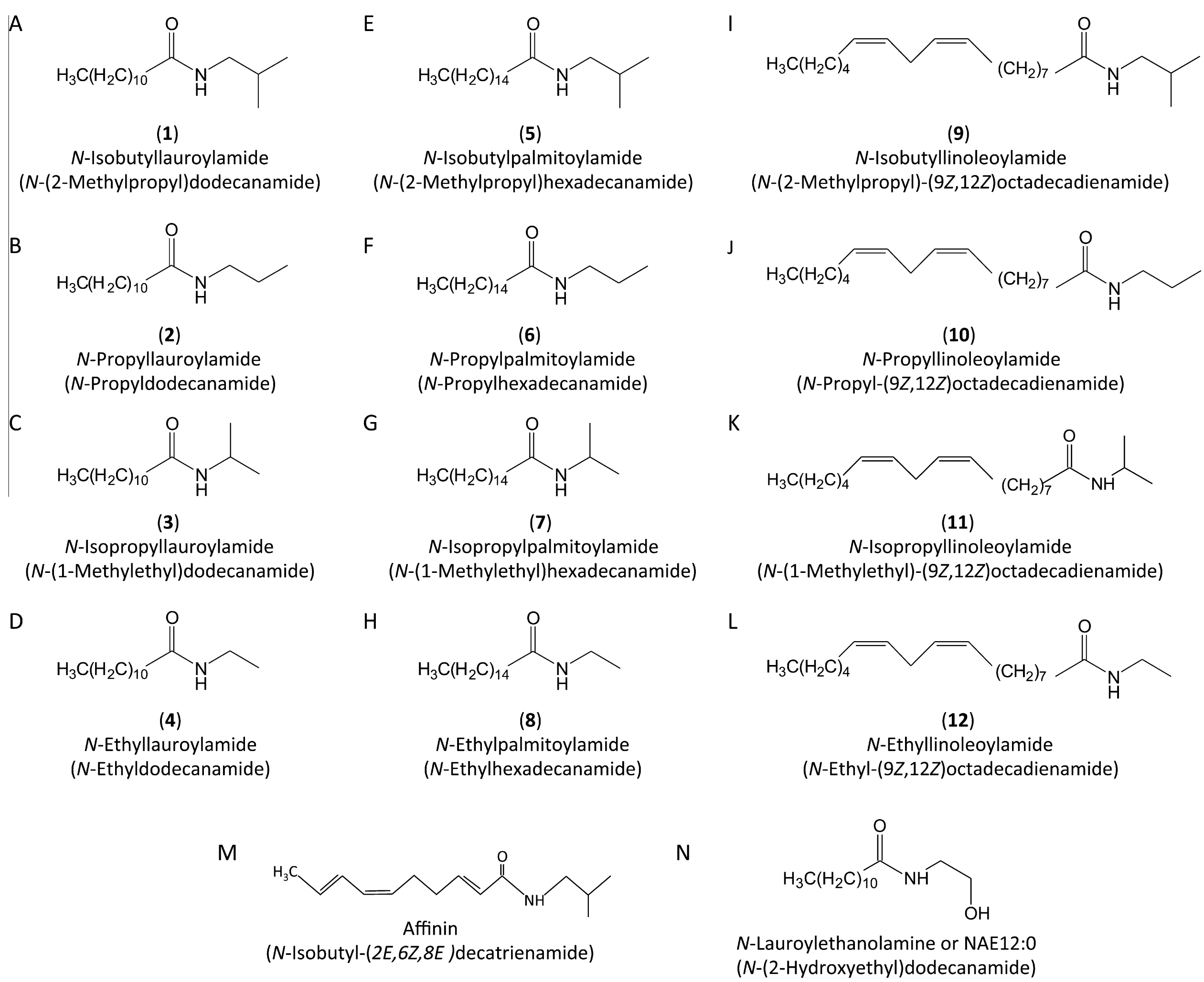

Fig. 1. Chemical Structure of "hybrid" alkamides. Three different categories of alkamide like compounds: (A–D) acyl chain with 12:0 (number of carbon atom:number of double bonds); (E–H) 16:0; (I–L) 18:2; (M) 10:3; (N) 10:0.

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