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Sensory active piperine analogues from *Macropiper excelsum* and their effects on intestinal nutrient uptake in Caco-2 cells

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ABSTRACT

The phytochemical profile of *Macropiper excelsum* (G.Forst.) Miq. subsp. *excelsum* (Piperaceae), a shrub which is widespread in New Zealand, was investigated by LC-MS-guided isolation and characterization via HR-ESI-TOF-MS and NMR spectroscopy. The isolated compounds were sensorily evaluated to identify their contribution to the overall taste of the crude extract with sweet, bitter, herbal and trigeminal impressions.

Besides the known non-volatile *Macropiper* compounds, the lignans (+)-diayangambin and (+)-excelsin, four further excelsin isomers, (+)-diasesartemin, (+)-sesartemin, (+)-episesartemin A and B were newly characterized. Moreover, piperine and a number of piperine analogues as well as *trans*-pellitorine and two homologues, kalecide and (2*E*,4*E*)-tetradecadienoic acid *N*-isobutyl amide were identified in *M. excelsum*, some of them for the first time. Methyl(2*E*,4*E*)-7-(1,3-benzodioxol-5-yl)hepta-2,4-dienoate was identified and characterized for the first time in nature. Sensory analysis of the pure amides indicated that they contributed to the known chemesthetic effects of *Macropiper* leaves and fruits. Since the pungent piperine has been shown to affect glucose and fatty acid metabolism *in vivo* in previous studies, piperine itself and four of the isolated compounds, piperdardine, chingchengenamide A, dihydropiperlonguminine, and methyl(2E,4E)-7-(1,3-benzodioxol-5-yl)hepta-2,4-dienoate, were investigated regarding their effects on glucose and fatty acid uptake by enterocyte-like Caco-2 cells, in concentrations ranging from 0.1 to 100 μM. Piperdardine showed the most pronounced effect, with glucose uptake increased by 83 ± 18% at 100 μM compared to non-treated control cells. An amide group seems to be advantageous for glucose uptake stimulation, but not necessarily for fatty acid uptake-stimulating effects of piperine-related compounds.

1. Introduction

Macropiper excelsum (G.Forst.) Miq. subsp. *excelsum* (= *Piper excelsum* G.Forst., Gardner, 1997), also called *Piper excelsum*, kawakawa, or New Zealand pepper tree, is a shrub belonging to the Piperaceae family (Jaramillo and Manos, 2001). *M. excelsum* is common in New Zealand, with predominant occurrence and distribution on the North Island (Dawson and Lucas, 2009). The orange, spadix-shaped fruits are part of the traditional Māori diet (Colenso, 1880). Besides the fruits, the aromatic, heart-shaped leaves are traditionally used as tea, spice, or to prepare the popular "Ti-toki" liqueur (Crowe, 2009). Kawakawa is also used as a traditional medicinal plant against bladder problems, gonorrhea, cuts, wounds, or against toothache (Crowe, 2007; Goldie, 1905; Armstrong, 1869).

The phytochemical profile of *M. excelsum* leaves has not yet been investigated in detail. Volatile compounds, myristicin (**14**), elemicin (**15**), and α-pinene, as well as some non-volatiles, such as the two lignans (+)-excelsin and (+)-diayangambin (**1**), have been described from this plant (Briggs, 1941; Briggs et al., 1975; Russell and Fenemore, 1973). Recently, a number of amides, piperine (**6**), piperdardine (**11**), chingchengenamide A (**12**), dihydropiperlonguminine (**13**), *cis*-/*trans*-fagaramid (**16**, **17**), ilepcimide (**18**), and Z-antiepilepsirine (**19**) were detected in the fruits of

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kawakawa (Lei et al., 2015), whereas information about their occurrence and distribution in other parts of the plant is still missing.

When chewing the leaves, a slightly irritating, numbing, or tingling sensation can be detected. Usually, this type of chemesthetic effect of plants is caused by trigeminal active compounds such as piperine, pellitorine (Patel et al., 1992), or spilanthol (Ley et al., 2006). In addition to their sensory impressions, these amides can exhibit antinociceptive and numbing effects, and may play a role in plant defense against environmental stress and herbivores (Crombie, 1955; Scott et al., 2003).

The traditional use of kawakawa as a natural health remedy may have its origin in already identified anti-inflammatory ingredients, such as the furofuran lignan (+)-diayangambin (**1**, De Leon et al., 2002) and myristicin (**14**, Lee and Park, 2011). Pellitorine is also known to exhibit anti-inflammatory effects *in vitro* and *in vivo* (Ku et al., 2014). Piperine (**6**), common in Piperaceae and also found in *M. excelsum*, has been associated with an increased glucose absorption from the gastrointestinal tract of rabbits (Patil et al., 2011), and with raised blood glucose levels after application of doses of 40 mg/kg in mice (Atal et al., 2012). Remarkably, application of the same dose also lowered blood lipids in mice fed a high-fat diet (Shah et al., 2011). These data suggest that piperine impacts intestinal fat and glucose absorption.

Lei et al. (2015) identified some piperine relatives in *M. excelsum*, but the knowledge on the phytochemical profile is still fragmentary. In addition, the compounds responsible for the chemesthetic effects have yet not been identified. Therefore, a thorough fractionation and isolation of fresh and dried leaves, seeds, and fruits of *M. excelsum* was performed. This was followed by sensory investigations of isolated compounds or their synthesized counterparts with special focus on the structural class of piperine derivatives. It is hypothesized that the compounds sharing structural core motifs with piperine may also influence intestinal macronutrient uptake. Here, we present the impact of selected amides and one ester related to piperine and isolated from *Macropiper* on glucose and fatty acid uptake in differentiated intestinal Caco-2 cells. Differentiated Caco-2 cells have been shown to be a suitable model for enterocytes and the intestinal barrier function, as they unfold typical characteristics like a brush border membrane and intracellular tight junctions (Pinto et al., 1983; Hidalgo et al., 1989).

2. Results and discussion

2.1. Identification of compounds in M. excelsum extract by comparison with reference molecules

Dried, cut leaves of *M. excelsum* were extracted with ethanol/water. The crude extract was analyzed via LC-MS; the respective chromatogram with assignments of the detected molecular masses is shown in Fig. 1. All identified compounds from *M. excelsum* are presented in Fig. 2. Myristicin (**14**) and elemicin (**15**), which were already described in the leaves of *M. excelsum* (Briggs, 1941; Briggs et al., 1975), were identified in the extract based on mass spectra. For the unambiguous identification of the compounds, comparisons of elution times as well as MS-MS fragments with those of reference compounds were carried out. In addition to these two compounds, a number of molecules with *N*-containing structures were detected. Taking into account literature data on related *Piper* species, it was suggested that these compounds belong to the structural class of amides. Based on this assumption, a selection of in-house available reference compounds, namely piperine (**6**), *trans*-pellitorine (**7**), *cis*-fagaramid (**16**), *trans*-fagaramid (**17**) and achilleamid (**20**) were analyzed via LC-MS. The resulting data were compared to the elution times and MS-MS spectra of the corresponding candidates in the extract, which all matched (Supplementary data, Table S1). So far, none of them have been described in the leaves of *M. excelsum*. However, the piperine derivatives **7**, **16**, and **17** have recently been identified in the fruits (Lei et al., 2015) and previously in various *Piper* species (Table 1).

The dried leaf extract was fractionated via fast centrifugal partition chromatography (FCPC), then preparative HPLC (pHPLC) was required for pure compounds. Several compounds could be identified based on literature data and comparison to reference compounds, but the structures of the remaining isolated compounds had to be elucidated by 1D and 2D NMR experiments (^{1}H, ^{13}C, ^{1}H,^{13}C gHSQC, ^{1}H,^{13}C gHMBC and ^{1}H,^{1}H gCOSY). Taking into account the molecular masses and the already identified *trans*-pellitorine (**7**), compounds **8**, with a molecular formula of $C_{16}H_{29}NO$, and **9**, with the molecular formula of $C_{18}H_{33}NO$, were assumed to be homologues with chain elongations by two and four methylene groups, respectively. The structures were elucidated by NMR spectroscopy, and verified by synthesis. Comparison of ^{1}H and ^{13}C NMR data of the isolated compound **8** and the data of the synthesized sample, as well as literature data (Abarbri et al., 1998) confirmed the structure to be kalecide (**8**). Since only a small amount of **9** was present in FCPC fraction 6, the candidate molecule, (2*E*,4*E*)-tetradecadienoic acid *N*-isobutyl amide, was synthesized as well. For the identification of **9** in the extract, elution time and MS-MS fragments of the synthesized material were compared to the trace compound. In addition, NMR data of the synthetic molecule **9** and data from literature (Abarbri et al., 1998) were compared. The data were congruent and thus an additional homologue of pellitorine could be identified.

Another compound isolated from fraction 6, with a molecular formula of $C_{15}H_{16}O_4$, could be identified as methyl (2E,4E)-7-(1,3-benzodioxol-5-yl)hepta-2,4-dienoate (**10**) by 1D and 2D NMR spectral information and comparison to the literature spectra of the parent carboxylic acid and the corresponding ethyl ester (De Araújo-Júnior et al., 2001). As **10** has only be described once in a patent application (Ley et al., 2015), the proposed compound was synthesized for confirmation, and the NMR data were compared with the isolated compound (supplementary data Fig. S1; Table S2).

From FCPC fractions 11–14, two molecules were isolated: **12** with the molecular formula of $C_{18}H_{23}NO_3$ and **11** with the molecular formula of $C_{19}H_{23}NO_3$. After 1D and 2D NMR analysis and comparison with data from literature, chingchengenamide A and piperdardine were identified. Synthetic samples were prepared for unambiguous identification. Recently, both have been identified in the fruits of *M. excelsum* (Lei et al., 2015).

Further molecules were isolated from FCPC fractions 17–22 of the *M. excelsum* extract. Compound **21** with a molecular formula of $C_{14}H_{17}NO$ was identified as *N*-(trans-cinnamoyl)piperidine, by comparing NMR data of the isolated compound with a synthesized sample and literature data (Leung et al., 2010).

Another amide, compound **19**, was also found in the leaves of *M. excelsum*. The structure was elucidated by NMR spectroscopy and was compared with data of the synthetic molecule and that found in literature from *P. capense* (Kaou et al., 2010). Based on the comparison of the ^{1}H and ^{13}C spectra, **19** was identified as (*Z*)-antiepilepsirine.

Another amide, **18**, was identified as ilepcimide, showing a molecular formula of $C_{15}H_{17}NO_3$. The NMR shifts are congruent to literature (Matsuda et al., 2009; Ee et al., 2009), with only small differences in the ^{13}C shifts in positions 12–14. This is due to the fact that the isolated sample was a mixture of compound **18** as the minor compound and **19**, which is an isomer of **18**. Thus, the chemical shifts were assigned to **18** from the 2D NMR data and not all multiplets could have been unambiguously assigned.

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