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Quantitation and bitter taste contribution of saponins in fresh and cooked white asparagus (*Asparagus officinalis* L.)

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ABSTRACT

A sensitive HPLC–MS/MS method was developed enabling the simultaneous quantification of bitter-tasting mono- and bidesmosidic saponins in fresh and processed asparagus (*Asparagus officinalis* L.). Based on quantitative data and bitter taste recognition thresholds, dose-over-threshold factors were determined for the first time to determine the bitter impact of the individual saponins. Although 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl]-(25*R*/*S*)-spirost-5-ene-3β-ol was found based on dose-over-threshold factors to be the predominant bitter saponin in raw asparagus spears, 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-{α-L-rhamnopyranosyl-(1 → 4)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*R*)-22-hydroxyfurost-5-ene-3β,26-diol, 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-{α-L-rhamnopyranosyl-(1 → 4)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*S*)-22-hydroxyfurost-5-ene-3β,26-diol, and (25*R*)- and (25*S*)-furost-5-en-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside were found as key bitter contributors after cooking. Interestingly, the monodesmosidic saponins **5a**/**b** were demonstrated for the first time to be the major contributor to the bitter taste of fresh asparagus spears, while the bidesmosides **1a**/**b** and **2a**/**b** may be considered the primary determinants for the bitter taste of cooked asparagus.

1. Introduction

Due to its unique flavour profile centring around well-balanced, sweet, sulfury and buttery notes, raw and cooked spears of white asparagus (*Asparagus officinalis* L.) are highly appreciated by consumers. Unfortunately, the attractive taste quality of bleached asparagus spears is hindered by a sporadic bitter off-taste, which is often the reason for negative consumer reactions and, therefore, causes a major problem for vegetable processors (Brückner, Geyer, & Ziegler, 2008; Brückner, Schwarzbach, & Schrödter, 2010).

Although a series of investigations suggested steroidal saponins to contribute to bitterness of asparagus (Brückner et al., 2010; Hostettmann & Marston, 1995; Kawano, Sakai, Sato, & Sakamura, 1975; Kawano, Sato, & Sakamura, 1977), recent application of an activity-directed sensomics approach succeeded in comprehensively mapping and identifying the molecular determinants of this bitter off-taste (Dawid & Hofmann, 2012a, 2012b). Among these bitter molecules, a series of mono- and bidesmotic saponins were found with low bitter taste recognition thresholds ranging between 10.9 and 199.7 μmol/L, namely 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-{α-L-rhamnopyranosyl-(1 → 4)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*R*)-22-hydroxyfurost-5-ene-3β,26-diol (protodioscin, **1a**), 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-{α-L-rhamnopyranosyl-(1 → 4)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*S*)-22-hydroxyfurost-5-ene-3β,26-diol (neoprotodioscin, **1b**), (25*R*)-furost-5-ene-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside ((25*R*)-ASP-II, **2a**), (25*S*)-furost-5-ene-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside (ASP-II, **2b**), (25*R*)-furostane-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside ((25*R*)-Dihydro-ASP-II, **3a**), (25*S*)-furostane-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside ((25*S*)-Dihydro-ASP-II, **3b**), 3-*O*-[{β-D-glucopyranosyl-(1 → 2)}{β-D-xylopyranosyl-(1 → 4)} β-D-glucopyranosyl] (25*S*),5β-spirostan-3β-ol (AS-1, **4**), 3-*O*-[{α-L-rhamnopyranosyl-(1 → 2)}{α-L-rhamnopyranosyl-(1 → 4)}-β-D-glucopyranosyl]-(25*S*)-spirost-5-ene-3β-ol (AS-2-I, **5a**), and 3-*O*-[{α-L-rhamnopyranosyl-(1 → 2)}{α-L-rhamnopyranosyl-(1 → 4)}-β-D-glucopyranosyl]-(25*R*)-spirost-5-ene-3β-ol (dioscin, **5b**) (Fig. 1). Recently, a positive correlation was reported between the total saponin concentration and the bitter taste of fresh asparagus spears (Brückner et al., 2010). In particular, protodioscin (**1a**) and methylprotodioscin (**6**) were highlighted as playing a major role in the bitter perception. In contradiction, other studies suggested methylprotodioscin as an artefact of the methanolic extraction, rather than a native phytochemical in fresh asparagus spears (Schwarzbach, 2004; Wang et al., 2003). In order to determine the taste contribution of the

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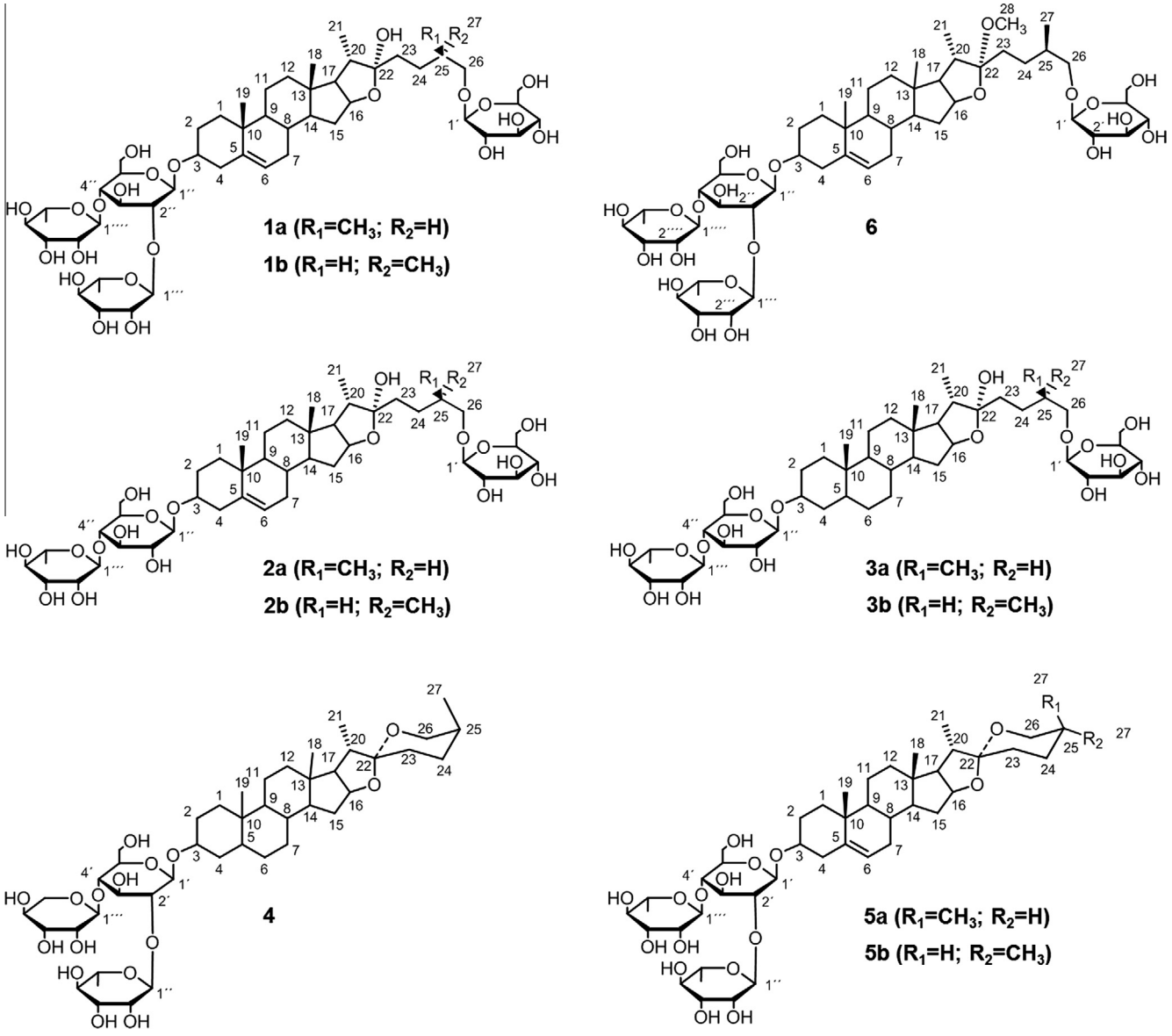

Fig. 1. Chemical structures of 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-{α-L-rhamnopyranosyl-(1 → 4)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*R*)-22-hydroxyfurost-5-ene-3β,26-diol (protodioscin, **1a**), 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-{α-L-rhamnopyranosyl-(1 → 4)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*S*)-22-hydroxyfurost-5-ene-3β,26-diol (neoprotodioscin, **1b**), (25*R*)-furost-5-en-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside ((25*R*)-ASP-II, **2a**), (25*S*)-furost-5-en-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside (ASP-II, **2b**), (25*R*)-furostane-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside ((25*R*)-Dihydro-ASP-II, **3a**), (25*S*)-furostane-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside ((25*S*)-Dihydro-ASP-II, **3b**), 3-*O*-[{β-D-glucopyranosyl-(1 → 2)}{β-D-xylopyranosyl-(1 → 4)}β-D-glucopyranosyl] (25*S*),5β-spirostan-3β-ol (AS-1, **4**), 3-*O*-[{α-L-rhamnopyranosyl-(1 → 2)}{α-L-rhamnopyranosyl-(1 → 4)}-β-D-glucopyranosyl]-(25*S*)-spirost-5-ene-3β-ol (AS-2-I, **5a**), 3-*O*-[{α-L-rhamnopyranosyl-(1 → 2)}{α-L-rhamnopyranosyl-(1 → 4)}-β-D-glucopyranosyl]-(25*R*)-spirost-5-ene-3β-ol (dioscin, **5b**) and 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-{α-L-rhamnopyranosyl-(1 → 4)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-22-methoxy-25(*R*)-furost-5-ene-3β,26-diol (methyl protodioscin, **6**).

individual saponins and, based on this knowledge, to target breeding programmes towards less bitter tasting asparagus varieties, analytical tools enabling an accurate and reliable quantitation of the key bitter drivers are urgently needed.

A series of attempts have been undertaken in recent years to quantitatively determine the contents of saponins, especially protodioscin (**1a**), in asparagus. The majority of quantitative saponin analyses were based on the use of high-performance thin-layer chromatography using post-chromatographic derivatisation with anisaldehyde-reagent (Brückner et al., 2010; Schwarzbach, 2004; Schwarzbach, Schreiner, & Knorr, 2006). Using this HPTLC approach, a total saponin content of 0.14–0.80 g/kg (fresh weight) in asparagus spears was reported (Schwarzbach et al., 2006). Alternatively, asparagus spears have been analysed for protodioscin by HPLC-UV, monitoring the effluent at 210 nm (Lee, Yoo, & Patil, 2010), but the lack of a suitable chromophore in most asparagus saponins is limiting their sensitive and accurate quantitation (Hostettmann & Marston, 1995). To overcome these challenges, HPLC–MS operating in the selected ion monitoring (SIM) mode was used for protodioscin quantitation (Wang et al., 2003). The authors found that the protodioscin content differed from the top, down to the bottom section of fresh asparagus shoots, from 0.24 up to 250 mg/100 g. However, this study neither targeted the quantitation of the entire set of the bitter key saponins **1a**/**b**–**5a**/**b** in asparagus, nor provided any information on the bitter taste contribution of the individual saponins.

The objective of the present investigation was, therefore, to identify the mono- and bidesmosidic saponins **1a**/**b**–**6** in different