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Antibacterial prenylated stilbenoids from peanut (*Arachis hypogaea*)

Wouter J.C. de Bruijn[a], Carla Araya-Cloutier[a], Judith Bijlsma[a], Anne de Swart[a], Mark G. Sanders[a], Pieter de Waard[b], Harry Gruppen[a], Jean-Paul Vincken[a,*]

[a] Laboratory of Food Chemistry, Wageningen University, P.O. Box 17, 6700 AA Wageningen, The Netherlands
[b] MAGNEFY (MAGNEtic Resonance Research FacilitY), Wageningen University, Wageningen, The Netherlands

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ABSTRACT

Stilbenoids are a class of secondary metabolites with a stilbene backbone that can be produced by peanut (*Arachis hypogaea*) as defence metabolites. Six monomeric prenylated stilbenoids, including the compound arachidin-6 (**4**), were isolated from extracts of fungus-elicited peanuts (*Arachis hypogaea*) using preparative liquid chromatography. Their structures were confirmed by MS^n, HRMS and NMR spectroscopy and their antibacterial activity was evaluated against methicillin-resistant *Staphylococcus aureus* (MRSA). Similarly to other phenolic compounds, prenylated derivatives of stilbenoids were more active than their non-prenylated precursors piceatannol, resveratrol, and pinosylvin. Chiricanine A (**6**), a chain-prenylated pinosylvin derivative, was the most potent compound tested, with a minimum inhibitory concentration (MIC) of 12.5 μg mL^{-1}. Arachidin-6 (**4**), a ring-prenylated piceatannol derivative, had moderate potency (MIC 50–75 μg mL^{-1}). In conclusion, prenylated stilbenoids represent a group of potential natural antibacterials which show promising activity against MRSA.

1. Introduction

Stilbenoids, a class of secondary metabolites with a stilbene backbone (Fig. 1A), can be produced by peanut (*Arachis hypogaea*) as defence metabolites (Sobolev, 2013). Analogous to phenolic metabolites of other members of the *Leguminosae* family, e.g. soy bean and mung bean (Aisyah et al., 2016; Simons et al., 2011), the production of stilbenoids and, in particular, their prenylated derivatives can be stimulated by fungal elicitation of germinating peanut seeds (Aisyah et al., 2015; Sobolev et al., 2010, 2016). Prenylation refers to the attachment of a prenyl-moiety (i.e. 3,3-dimethylallyl) by a prenyltransferase and in the case of peanut stilbenoids occurs mainly at the 4-position (Yang et al., 2016) as in, for example, arachidin-2 (Fig. 1B, **compound 2**) (Sobolev et al., 2016). Prenylation of phenolic compounds has been shown to increase their antibacterial activity, which is exemplified by the minimum inhibitory concentrations (MICs) of genistein (MIC > 128 μg mL^{-1}), 6-prenyl-genistein (MIC 32 μg mL^{-1}), and 6,8-diprenyl-genistein (MIC 8 μg mL^{-1}) against methicillin-resistant *Staphylococcus aureus* (MRSA) (Hatano et al., 2000). More generally, prenylated (iso) flavonoids have been shown to possess antibacterial activity against antibiotic-resistant strains of *S. aureus* and other pathogenic gram-positive bacteria (Araya-Cloutier et al., 2017, 2018a; Gibbons, 2004). An extract from fungus (*Rhizopus*) elicited peanut seedlings, enriched in prenylated stilbenoids, already showed promising antibacterial activity against *E. coli*, L. *monocytogenes* and MRSA (Araya-Cloutier et al., 2017, 2018b). In this study, we have isolated and characterized several prenylated compounds with different stilbenoid precursors and prenyl configurations from an extract of *Rhizopus*-elicited peanut seedlings and assessed their antibacterial activity against MRSA. In analogy with other phenolic compounds, we hypothesize that prenylation of stilbenoids will enhance their antibacterial activity.

2. Results and discussion

2.1. Sample clean-up, pre-purification and purification by preparative RP-HPLC

The crude extract of *Rhizopus*-elicited peanut seedlings showed a chromatographic profile on RP-UHPLC comparable to what was described previously (Aisyah et al., 2015). The clean-up with ethyl acetate effectively removed the majority of polar impurities in the extract, yielding the cleaned extract which contained mainly prenylated stilbenoids (Fig. S1, Supplementary data, 6–19 min) and apolar impurities (Fig. S1, 19-28 min) which, based on LC–MS analysis, were mostly lipids like oxylipins and free fatty acids (Murphy, 2014). After pre-purification by Flash chromatography, most of the apolar impurities

* Corresponding author.
E-mail address: jean-paul.vincken@wur.nl (J.-P. Vincken).

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Table 1
Spectrometric and spectroscopic data of purified compounds as determined by UHPLC-PDA coupled to ESI-IT-MS and ESI-FTMS.

Compound[a]	λ_{max} (nm)	Ionisation	IT-MS		Molecular formula	*m/z* calc.	FTMS	
			m/z precursor	MS^2 product ions (relative abundance)[b]			*m/z* obs.[c]	Error (ppm)
1	339	$[M-H]^-$	311	241, 242 (93), 312 (76), 255 (46), 267 (45), 311 (31), 293 (25), 224 (20), 172 (16)	$C_{19}H_{20}O_4$	311.12888	311.12907	0.60
		$[M+H]^+$	313	257				
2	322	$[M-H]^-$	295	239, 296 (55), 240 (43), 226 (42), 295 (38)	$C_{19}H_{20}O_3$	295.13397	295.13388	−0.30
		$[M+H]^+$	297	241				
3	338	$[M-H]^-$	295	239, 240 (48), 226 (31), 295 (26), 227 (25), 251 (18)	$C_{19}H_{20}O_3$	295.13397	295.13402	0.18
		$[M+H]^+$	297	241				
4	342	$[M-H]^-$	309	309, 310 (63), 265 (60), 291 (24), 294 (21), 281 (18)	$C_{19}H_{18}O_4$	309.11323	309.11334	0.35
		$[M+H]^+$	311	201, 283 (55), 135 (50), 187 (40), 177 (30), 293 (29), 123 (28), 269 (25), 175 (22), 183 (20), 173 (19), 265 (17), 189 (16), 202 (16)				
5	339	$[M-H]^-$	293	293, 278 (47), 294 (35)	$C_{19}H_{18}O_3$	293.11832	293.11847	0.52
		$[M+H]^+$	295	201, 267 (93), 253 (51), 175 (37), 107 (37), 277 (32), 183 (28), 225 (24), 239 (23), 173 (19), 159 (18), 119 (18), 249 (15)				
6	312	$[M-H]^-$	279	224, 223 (85), 279 (66), 280 (50), 211 (15)	$C_{19}H_{20}O_2$	279.13905	279.13914	0.31
		$[M+H]^+$	281	225				

[a] Data provided based on the *trans* isomer.
[b] Most abundant fragment is underlined, only fragment ions with a relative abundance of at least 15 are shown.
[c] Based on the average of 5 spectra in negative ionisation mode.

were removed and several pools were obtained enriched in mixtures of prenylated stilbenoids and some oxylipins (Fig. S1). After subjecting these pools to preparative RP-HPLC separation, six purified compounds were obtained.

2.2. Structure elucidation of the prenylated stilbenoids

The six compounds that were isolated were first analysed by UHPLC-PDA-ESI-IT-MS, the corresponding spectrometric and spectroscopic data of which is shown in Table 1. Based on the comparison of this data to literature (Aisyah et al., 2015; Sobolev et al., 2006, 2009), the compounds were tentatively annotated. For most purified compounds, the *trans* isomer was most abundant but the *cis* isomer was also present. The two isomers were distinguished by their λ_{max}, and the peak with the higher λ_{max} was assigned as the *trans* isomer, in accordance with previously reported data (Trela and Waterhouse, 1996). Based on UV_{310} area compound **1** was approximately 60% *trans*, compound **3** was approximately 88% *trans*, and compounds **2**, **4**, **5** and **6** were all more than 97% *trans*. Spectrometric and spectroscopic data provided is based on the *trans* isomer.

In ESI-IT-MS (Table 1), the most abundant fragments observed for compounds **1**-**3** and **6** in negative ionisation mode were those with a neutral loss (NL) of 56 u (C_4H_8) and NL of 69 or 70 u, corresponding to complete loss of the prenyl chain (as C_5H_8 or C_5H_9). In positive ionisation mode the main fragments were also related to the prenyl-moiety, resulting in a NL of 56 u (C_4H_8), as described previously for chain-prenylated (iso)flavonoids (Simons et al., 2009). For compounds **4** and **5** the main fragment observed in positive ionisation mode, *m/z* 201, corresponded to loss of the catechol (NL 110 u, $C_6H_4O_2$) or phenol (NL 94 u, C_6H_4O) moiety, respectively. Fragments corresponding to neutral losses of 56 u and 42 u were also observed. For compound **4** the fragment at *m/z* 269 (NL 42 u, rel. abundance 25) was more intense than the fragment at *m/z* 255 (NL 56 u, rel. abundance 13, not shown in Table 1). The same was observed for compound **5** with the fragments at *m/z* 253 (NL 42 u, rel. abundance 51) and *m/z* 239 (NL 56 u, rel. abundance 23). The abundance ratio of NL 42:56 u was > 1 for both compounds, indicating the presence of a ring prenyl rather than a chain prenyl (Simons et al., 2011). In negative ionisation mode, the prenyl-moiety of compounds **4** and **5** did not readily fragment, instead the unfragmented parent ion, radical fragments and small neutral losses like $\bullet CH_3$ (15 u), H_2O (18 u), CO (28 u), and CO_2 (44 u) were observed.

High resolution mass spectrometric data, as determined by UHPLC-ESI-FTMS, confirmed the expected molecular formulae of all six compounds (Table 1). To confirm the tentative annotations of the structures, ^1H NMR spectra of compounds **1**-**6** were acquired. The structure of compounds **1**-**3**, **5** and **6** was confirmed by comparison of their ^1H NMR spectra to published data (Chang et al., 2006; Park et al., 2011; Royer et al., 2010; Sobolev et al., 2009). Compound **4** ($C_{19}H_{18}O_4$ based on FTMS), however, which was previously tentatively annotated as 4-isopentadienyl-3,5,3′,4′-tetrahydroxystilbene (IPP) based on UHPLC-PDA-ESI-IT-MS (Aisyah et al., 2015), did not match this compound's expected ^1H NMR spectrum. HMBC and HMQC were performed in order to elucidate the structure of compound **4** (see Table 2 for the ^1H and ^{13}C NMR spectroscopic data). The ^{13}C NMR spectrum showed signals identical to those described for the catechol moiety of arachidin-1 (Chang et al., 2006). These signals were thereby assigned as aromatic carbons C-1′ to C-6′. Based on the HMBC and HMQC cross peaks of these carbons, the three ^1H NMR signals at δ_H 6.974 (d, J = 2.0 Hz, H-2′), 6.737 (d, J = 8.2 Hz, H-5′), and 6.835 (dd, J = 8.2 and 2.0 Hz, H-6′) were assigned as the corresponding protons. The olefin carbons C-α (δ_C 126.75) and C-α′ (δ_C 129.69) showed HMQC cross peaks with two doublet proton signals at δ_H 6.721 (J = 16.2 Hz, H-α) and 6.889 (J = 16.2 Hz, H-α′), whose coupling constants confirmed the *trans*-olefin. Both of these protons showed cross peaks with aromatic carbons C-1′ and C-1 (δ_C 140.23). Proton H-α also showed HMBC cross peaks