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A DIENYL STILBENE PHYTOALEXIN FROM *ARACHIS HYPOGAEA*

CHRISTOPHER J COOKSEY, PETER J. GARRATT, SUSAN E RICHARDS* and RICHARD N STRANGE*

Department of Chemistry, University College London, 20 Gordon Street, London WC1H OAJ, U K., *Department of Botany and Microbiology, University College London, Gower Street, London WC1E 6BT, U K

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Abstract—The novel stilbene, 3-isopentadienyl-4,3',5'-trihydroxystilbene, was identified as the major antifungal component elicited by slicing imbibed kernels of 11 genotypes of groundnut Incubation for 24 hr after slicing yielded values which varied between 38 5 and 105 8 μg of the compound/g of unimbibed kernels according to cultivar. After incubation for 48 hr, yields rose to between 89 5 and 189.7 μg/g. The compound was inhibitory to both spore germination and hyphal extension of the fungus, *Aspergillus flavus*, at 14 0 and 11.3 μg/ml, respectively

INTRODUCTION

We previously showed that wounding by slicing was an effective stimulus of phytoalexin accumulation in groundnut kernels [1]. We now report that a new stilbene phytoalexin is the major antifungal component of groundnut kernels of 11 genotypes treated in this manner and that smaller amounts of resveratrol were present as well

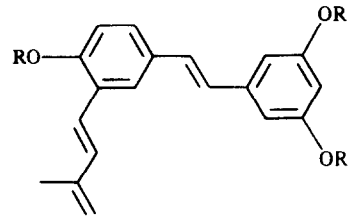
RESULTS

The major component, **1a**, was isolated by reverse phase semi-preparative HPLC The mass spectrum gave *m/z* 294 1243 ($C_{19}H_{18}O_3$ requires 294 1255) and there were prominent fragment ions at 279 $[M-15]^+$ and 228 $[M-66]^+$ The ^{1}H NMR spectrum (acetone-d_6) showed signals at δ7 75 (*d*, 1H, J=2.2 Hz), 7 33 (*dd*, 1H, J=8.4, 2 1 Hz) and 6 90 (*d*, 1H, J=8.4 Hz), doublets at δ7.11 (1H, J=16 4 Hz) and 6.93 (1H, J=16 4 Hz), doublets at δ7 06 (1H, J=16.5 Hz) and 6 94 (1H, J=16.5 Hz), a doublet at δ6.56 (2H, J=2 1 Hz), a triplet at δ6 28 (1H, J=2 1 Hz), one proton multiplets at δ5.14 and 5.07 and a double doublet at δ1 98 (3H, J=0 7, 1.4 Hz). Selective irradiation of the proton at δ1 98 collapsed the multiplets at δ5 14 and 5 07 to an AB quartet (J=2.4 Hz). Irradiation of the proton at δ5 14 collapsed the signal at δ1.98 to a doublet (J=1.6 Hz), and irradiation of the proton at δ5.07 collapsed the signal at δ1.98 to a broad singlet Methylation gave **1b**. The mass spectrum gave *m/z* 336 with the most intense ion at 270 $[M-66]^+$. The ^{1}H NMR spectrum ($CDCl_3$) showed signals at δ7.66 (*d*, 1H, J=2 2 Hz), 7.36 (1H, *dd*, J=8 4, 2.2 Hz) and 6.85 (1H, *d*, 8 4 Hz), two AB quartets at δ6 98 and 6.94 (J=16.2 Hz), a doublet at δ6.66 (2H, J=2.2 Hz) and a triplet at δ6.37 (1H, J=2 2 Hz), one proton multiplets at δ5.13 and 5 07, singlets at δ3.95 (3H) and 3 82 (6H), and a broad singlet at δ2.00 (3H). An NOE experiment showed that irradiation of the signal at δ3 82 enhanced the signals at δ6.66 and 6 37. The electronic spectrum (methanol) of **1a** showed an absorption maximum at 296 nm which shifted to give a broad peak at 341 nm on addition of NaOH

The concentrations of resveratrol and **1a** for the 11 genotypes varied considerably, although the yields of resveratrol were always less than **1a** at both incubation times (Table 1). The three stilbene phytoalexins previously described [2] accumulated in most of the genotypes in response to wounding but their concentrations were typically in the same range as those of resveratrol. The ED_{50} values of inhibition of spore germination and hyphal extension of *Aspergillus flavus* were 14.0 and 11.3 μg/ml, respectively.

DISCUSSION

Ingham [3] and Keen and Ingham [4] have reported that kernels of American groundnuts accumulated *cis*- and *trans*-4-isopentenylresveratrol whereas those of the African groundnut accumulated *cis*- and *trans*-resveratrol. Subsequently, we showed that American groundnut kernels also accumulate two other stilbene phytoalexins [2]. The major phytoalexin of leaves of the plant, synthesized in response to infection by two leaf spot fungi, was the pterocarpan phytoalexin, medicarpin [5] In this paper we report on the kernel phytoalexins of 11 defined genotypes of the plant. In contrast to the earlier work, we have found that the predominant phytoalexin, although a stilbene, has the isoprenyl group in the less hydroxylated ring and that this group is doubly unsaturated. At present, it is not clear to us why this compound was not recognized previously [2–4].



1a R = H
1b R = Me

Table 1 Yield of resveratrol and compound **1a**

	Amount (μg/g unimbibed kernels)			
	Resveratol		**1a**	
Genotype	24 hr*	48 hr	24 hr	48 hr
A 7404	20 1 ± 3 5†	31 9 ± 6 3	105 8 ± 14 1	157 5 ± 28 0
A 7717	18 2 ± 4 6	37 4 ± 4 8	84 9 ± 9 1	140 0 ± 24 7
A 7405	23 8 ± 6 5	34 7 ± 6 0	78 0 ± 14 3	124 0 ± 26 6
PI 337394F	15 0 ± 5 8	42 2 ± 8 3	80 4 ± 13 0	151 2 ± 24 9
PI 337458	19 1 ± 2 3	33 1 ± 5 7	71 8 ± 15 2	189 7 ± 32 5
A 7223	17 4 ± 1 7	25 0 ± 4 4	60 0 ± 19 3	119 7 ± 32 0
A 7293	10 4 ± 1 8	21 2 ± 5 5	65 4 ± 23 2	133 6 ± 23 8
PI 337409	15 8 ± 2 5	29 7 ± 4 6	70 1 ± 8 7	126 9 ± 13 8
A 7309	7 9 ± 3 3	23 8 ± 6 1	51 0 ± 11 5	101 8 ± 17 5
Florunner	4 3 ± 3 1	33 7 ± 6 6	40 5 ± 9 6	159 5 ± 18 3
PI 331326	5 9 ± 3 7	22 5 ± 2 9	38 6 ± 6 1	89 5 ± 21 3

*Incubation time
†Mean of five replicates ± s d

We have recently shown that the occurrence of phytoalexins in groundnut kernels is of potential significance as a defence response against mycotoxigenic fungi of the *Aspergillus flavus* group [1, 6] Work is now in progress to determine the role of resveratrol and **1a** as defence compounds in this interaction

EXPERIMENTAL

Elicitation and extraction of phytoalexins Batches (5 g) of groundnut kernels of 11 genotypes designated in Table 1 were surface sterilized for 15 min in 20% H_2O_2 and rinsed in sterile distilled H_2O After soaking for 12 hr in sterile distilled H_2O, the testas were removed and the kernels cut into slices *ca* 2 mm thick The sliced kernels were incubated for 24 or 48 hr at 25° in the dark Phytoalexins were extracted by the facilitated diffusion technique [7] with MeCN as solvent After vacuum infiltration, the sliced kernels were soaked in the solvent for 24 hr The extract was diluted to 30% MeCN with H_2O and applied to a cartridge consisting of a 1 ml Gilson pipette tip containing ODS silica (0 5 g, Techoprep 25/40 μ) The ODS silica was kept in place by a plug of glass wool at each end After washing with 30% MeCN (3 ml), the phytoalexins were eluted in 100% MeCN The vol of the eluate was adjusted to 5 ml

Separation of the phytoalexins by HPLC Samples (0 2 ml) were injected onto a column (25 × 1 0 cm i d) of Spherisorb 10 ODS The column was eluted with 50% MeCN and the HPLC detector was set at 290 nm Active fractions were initially defined by their ability to inhibit *Cladosporium herbarum* in a TLC assay [8] and subsequently by their R_f and absorption of light at 290 nm

Quantitative analysis of phytoalexins HPLC analysis was performed on a column (250 × 4 6 mm i d) packed with Spherisorb 10 ODS Samples (10 μl) were eluted in a solvent gradient consisting of A, HOAc 1% and B, MeCN The % B was increased linearly from 40–45% over the first 7 min, held at 45% for 5 min and then increased linearly to 65% over the final 8 min The flow rate was 1 5 ml/min and the eluant was monitored with a multichannel detector set at 310 nm and 0 04 A Concentrations of the phytoalexins were determined by reference to peak areas of standard preparations of the compounds An extinction coefficient of log 4 3425 at $\lambda = 298$ nm was assumed for **1a** in the preparation of standards of this compound

Antifungal assays Resveratrol and **1a** were assayed for their effects on spore germination and hyphal extension essentially as previously described [1]

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