not possible either. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-140363 (**1**) and CCDC-140364 (**2a**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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Channel-Forming Peptaibols Are Potent Elicitors of Plant Secondary Metabolism and Tendril Coiling**

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Attack by microorganisms and herbivorous insects may induce characteristic local and systemic responses in plants. Typical defense reactions include de novo biosynthesis of phytoalexins and, characteristic of insect damage, the emission of volatile compounds for long-distance interactions.[1, 2] The molecular basis for recognition of either infection or herbivore interference at the plant's cell surface, however, is not well understood. The signal chain is often triggered by compounds from the attacking organism, generally referred to as elicitors. Subsequent intracellular signal transduction involves changes in the ion permeability of the plasma membrane,[3] which initiates a complex network of intracellular events leading to an increase of biosynthetic activities. The involvement of ion channels in signaling was previously demonstrated with parsley cells and an oligopetide elicitor,[4] with tobacco cells and oligogalacturonides,[5] and, more recently, with several proteinaceous elicitors involved in phytoalexin biosynthesis in plants.[6]

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In addition to macromolecular elicitors, certain fungi also produce low molecular weight peptide-type antibiotics with membrane-depolarising properties.[7] Characteristic features of their chemical structures are the acylated N terminus, and the presence of α-aminoisobutyric acid (Aib) and C terminal α-amino alcohols (peptaibols). They occur mainly as large 18- to 20-membered peptaibols, such as alamethicin,[7] ampullosporin[8] and chrysospermin,[9] and as smaller 15- to 16-membered species, such as antiamoebin.[10] Their antimicrobial activity is largely due to the formation of α-helical structures which produce voltage-dependent or voltage-independent ion channels within biological membranes.[7, 11] Since the induction of phytoalexin biosynthesis in plants may be directly linked to ion fluxes and subsequent intracellular signaling,[3, 4] this property of the peptaibols prompted us to study their potential effect on the secondary metabolism of plants.

Here we demonstrate for the first time that fungal peptaibols represent a novel and powerful class of elicitors that can induce multiple metabolic activities such as ethylene emission, biosynthesis of volatile substances, and tendril coiling.

As a representative model compound we chose alamethicin (ALA), a mixture of homologous peptaibols from the wide spread soil fungus *Trichoderma viride*, the major component of which contains eight Aib residues and two prolines.[7, 11] The N terminus is acetylated, and the C-terminal residue is phenylalaninol (Table 1). ALA is capable of forming voltage-gated channels which exhibit very high conductance in lipid membranes.[7, 11]

When ALA (5 μM) was supplied to the shoots of young Lima bean plants (*Phaseolus lunatus*) through the transpiration stream,[12] subsequent monitoring of the surrounding gas phase in a continuous flow system by photoacoustic spectroscopy[13, 14] revealed the emission of ethylene as one of the plant's earliest responses to the peptaibol (Figure 1a). The emission of ethylene started approximately 3 h after the onset of the stimulus, reached a transient maximum after about 7.5 h and leveled off during the following 5 h; this response resembles the profile of ethylene emission after induction with the proteinaceous elicitor cellulysin[14] or with jasmonic acid.[15] Continued monitoring of the gas phase by absorption of emitted volatile compounds onto activated carbon in a closed system,[12] followed by desorption and mass spectrometric analysis of the trapped compounds, demonstrated that the biosynthesis of certain terpenoids and aromatic components had also been increased by the treatment with ALA. The gas chromatogram (Figure 1b) shows that DMNT (5%) and methyl salicylate (MeSA) (4%) are the main compounds formed after TMTT (91%). Interestingly, the same profile of terpenoids can be induced by treatment of Lima bean leaves with 12-oxophytodienoic acid (12-OPDA),[12] an early biosynthetic precursor of jasmonic acid (JA). JA itself, provokes a more complex pattern of volatile compounds when applied to Lima bean leaves.[15] The induction of volatile compound biosynthesis by ALA was dose dependent with a threshold concentration in the range of approximately 0.5 μM.

Owing to the presence of MeSA in the gas phase and the induction of biosynthesis of terpenoids, which represent a

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Table 1. Peptaibols and peptides as elicitors of volatile compound production.

Peptaibols and peptides[12]	volatile compound biosynthesis (in *P. lunatus*)
alamethicin F[7][a]	
Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Pheol	+
ampullosporin A[8]	
Ac-Trp-Ala-Aib-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Gln-Leu-Aib-Gln-Leuol	+
bergofungins[25]	
A: Ac-Val-Aib-Aib-Aib-Val-Gly-Leu-Aib-Aib-Hyp-Gln-iVal-Hyp-Aib-Pheol	+
B: Ac-Val-Aib-Aib-Aib-Val-Gly-Leu-Val-Aib-Hyp-Gln-iVal-Hyp-Aib-Pheol	+
C: Ac-Val-Aib-Aib-Aib-Val-Gly-Leu-Aib-Aib-Hyp-Gln-Aib-Hyp-Aib-Pheol	+
chrysospermin A[9][b]	
Ac-Phe-Aib-Ser-Aib-Aib-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib-Pro-Aib-Aib-Aib-Gln-Trpol	+
melittin[21]	
Gln-Gln-Arg-Lys-Arg-Lys-Ile-Try-Ser-Ile-Leu-Ala-Pro-Leu-Gly-Thr-Thr-Leu-Val-Lys-Leu-Val-Ala-Gly-Ile-Gly	–
valinomycin[22]	
Cyclo[-L-Val-D-Hylva-D-Val-L-Lac]$_3$	–
bradykinin[22]	
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Argol	–
systemin[24]	
Ala-Val-Gln-Ser-Lys-Pro-Pro-Ser-Lys-Arg-Asp-Pro-Pro-Lys-Met-Gln-Thr-Asp	–
substance P[23]	
Met-Leu-Gly-Phe-Phe-Gln-Gln-Pro-Lys-Pro-Arg	–

[a] Commercial alamethicin is a mixture of homologous peptides.[7, 12] [b] A mixture of chrysospermins A – D was applied. Aib = 2-Amino-2-methylpropionic acid, Argol = argininol, Hyp = hydroxyproline, Hylva = α-hydroxyisovalerianic acid, Lac = lactic acid, Leuol = leucinol, Pheol = phenylalaninol, Trpol = tryptophanol. Amino acids have L-configuration unless specified otherwise.

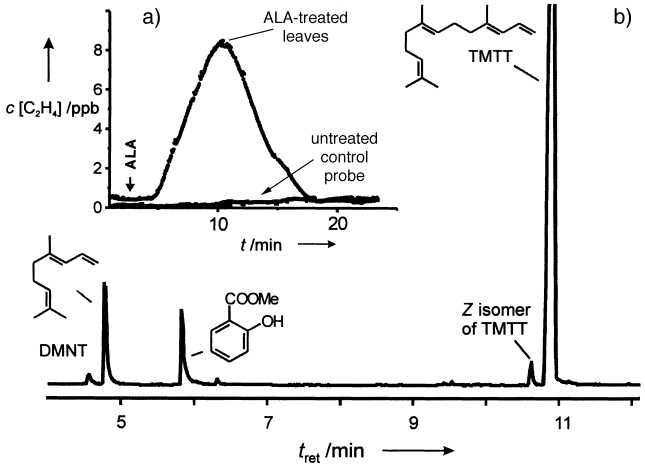

Figure 1. Profile of emitted volatile compounds from Lima bean leaves (*P. lunatus*) after treatment with alamethicin (ALA). Ethylene was determined in a continuous flow system by photoacoustic spectroscopy.[13] a) Time course of ethylene emission. b) Gas chromatographic profile of other volatile compounds. The intensity of the signals is enhanced to show minor compounds. Quantitative composition of the blend: 4,11-dimethylnona-1,3,7-triene (DMNT), 5%; methyl salicylate (MeSA), 4%; 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT), 91%. No volatile compounds were released from the untreated control plants.

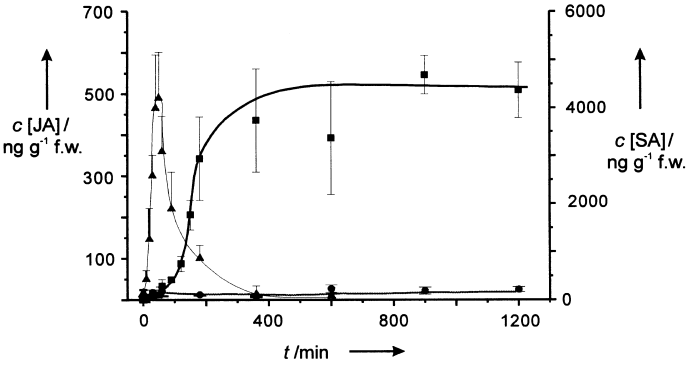

Figure 2. Quantity of endogenous jasmonate and salicylate produced over time in Lima bean leaves after treatment with ALA. ■ = Salicylate (SA), ▲ = jasmonate (JA), ● = SA or JA in untreated leaves (f.w. = fresh weight).

typical JA-inducible class of volatile compounds, we next quantified the endogenous levels of SA and JA in ALA-treated leaves.[16] As shown in Figure 2, both signaling pathways were activated. The typical early and transient production of JA (approximate 20-fold increase within 80 min), together with a steady and massive production of SA which began after 2 h and reached a plateau after 6 h (approximate 90-fold increase) was observed. Control plants, without ALA treatment, showed no volatile-compound production and did not exhibit raised levels of endogenous SA or JA. If the leaves were treated with inhibitors of the octadecanoid pathway (such as phenidone[17] or aristolochic acid[18]) prior to induction with ALA, no volatile compounds were emitted. Since inhibitor-treated plants can start to produce such compounds again after exogenous application of JA, the involvement of the octadecanoid signaling pathway was unambiguously corroborated.

The biological activity of ALA is not restricted to Lima bean plants, but appears to be more general. The phylogenetic archetype fern (*Dryopteris filix-mas*) responds with a very pronounced emission of a complex pattern of sesquiterpenoids.[15] The same class of compounds dominates in the spectrum of ALA-treated Mung bean (*Vigna radiata*), cotton (*Gossypium hirsutum*), or maize (*Zea mays*) plants.[2, 15] The garden bean (*Phaseolus vulgaris*) showed a profile comparable to that of the Lima bean (similar to Figure 1). Soy bean (*Glycine max*) and garden pea (*Pisum sativum*) plants failed to produce volatile compounds.

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The increase of JA production in the Lima bean plants prompted us to test ALA in tendril-coiling experiments. Tendrils of *Bryonia dioica* respond to JA, MeJA, and 12-OPDA with a coiling reaction comparable to the free coiling reaction of mechanically stimulated tendrils.[19] Tendrils of *Bryonia dioica*, *Pisum sativum*, and *Lathyrus* sp. were treated with solutions of ALA (5 μM) through the transpiration stream and, after 20 h, the degree of coiling was measured. Alternatively, tendrils were subjected to the coiling assay described in the literature.[20] All tested plant species exhibited a rapid coiling response towards ALA. Surprisingly, inhibition of the octadecanoid cascade with phenidone did not hamper the coiling reaction, which indicates that signaling systems independent of the lipid-based pathway, such as ion fluxes, have to be considered as elements of mechanotransduction.[20]

Besides ALA, many other peptaibols and peptides endowed with pore-forming or ion-transporting properties are known. To establish whether pore formation within a membrane or ion transport through a membrane is the underlying principle for the induction of volatile compound biosynthesis, other pore-forming peptaibols and some typical ion transporters were tested. The results are compiled in Table 1. The entire group of peptaibols induced the same pattern of volatile compounds in Lima bean leaves, suggesting a common mode of action. Although the basic peptide melittin is known as a pore-forming compound,[21] it failed to induce volatile biosynthesis in Lima bean leaves. A typical ion transporter, K^+ selective valinomycin[22] (9 μM), was also found to be inactive. Other biologically active small peptides which act through specific receptors, such as the undecapeptide "substance P",[23] the nonapeptide bradykinin,[22] and systemin, a signal peptide of tomato plants,[24] did not induce volatile compound biosynthesis, supporting the pore-forming capability of peptaibols as the essential property for the elicitation process.

Channel-forming peptides, such as alamethicin, may be used as valuable tools to unravel the early events of plant defense under well defined conditions. First analyses of insect salivary secretions have already demonstrated the presence of pore-forming compounds,[26] suggesting that membrane depolarisation also has to be considered as an important element of insect-induced plant defense.

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Sol – Gel Polycondensation of Tetraethoxysilane in a Cholesterol-Based Organogel System Results in Chiral Spiral Silica

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Exploitation of new organic gelators that can gelate various organic solvents has become an active area of research.[1–11] These organogels are of particular interest because they are different from polymer gels. Fibrous aggregates of low molecular weight compounds formed by noncovalent interactions are responsible for such gelation phenomena. Hence, the xerogels exhibit various superstructures, reflecting the monomeric structure of each gelator. This is why the study of organogels is considered to be a new field of supramolecular chemistry.[11]

Recently, it was found that certain cholesterol derivatives can gelate even tetraethoxysilane (TEOS), which results in

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