the active site of ODCase must provide two features: a low dielectric interior to allow for pK_a tuning so that the catalytic proton transfer to O-4 can occur, and a perfectly placed lysine to effect that proton transfer to O-4, concerted with decarboxylation.

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31. Solvation was modeled with the SCI-PCM method, available in Gaussian 94 (*16*). Solvation energies were obtained by calculating RHF/6-31+G* energies in solvent of a given dielectric and comparing that value to the gas-phase energy. These solvation energies were then used to correct MP2/6-31+G* energies to obtain $\Delta H^{\ddagger}$ values in solution.

32. We are grateful to NSF and NIH (postdoctoral fellowship to J.K.L.) for financial support of this work and to the UCLA Office of Academic Computing and the National Center for Supercomputing Applications for computational resources.

16 December 1996; accepted 19 March 1997

An Elicitor of Plant Volatiles from Beet Armyworm Oral Secretion

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The compound *N*-(17-hydroxylinolenoyl)-L-glutamine (named here volicitin) was isolated from oral secretions of beet armyworm caterpillars. When applied to damaged leaves of corn seedlings, volicitin induces the seedlings to emit volatile compounds that attract parasitic wasps, natural enemies of the caterpillars. Mechanical damage of the leaves, without application of this compound, did not trigger release of the same blend of volatiles. Volicitin is a key component in a chain of chemical signals and biochemical processes that regulate tritrophic interactions among plants, insect herbivores, and natural enemies of the herbivores.

The intriguing defensive reaction of plants, whereby plant volatiles induced by insect herbivore injury attract natural enemies of the herbivores, is triggered when a substance in the oral secretion of the insect herbivores contacts damaged plant tissue. We have isolated, identified, and synthesized *N*-(17-hydroxylinolenoyl)-L-glutamine (Fig. 1), which we have named volicitin, from the oral secretion of beet armyworm (BAW) (*Spodoptera exigua* Hübner) caterpillars. Synthesized and natural volicitin induce corn (*Zea mays* L.) seedlings to release the same blend of volatile terpenoids and indole released when they are damaged by caterpillar feeding (*1*). This blend of volatile compounds attracts females of the parasitic wasp *Cotesia marginiventris*, natural enemies of BAW caterpillars, to the damaged corn plants (*2*).

The similarity of the structure of the insect-produced elicitor with the structures of the precursors of eicosanoids and prostaglandins involved in signaling in insects and other animals (*3*) and the components of the octadecanoid signaling pathway in plants (Fig. 1) (*4*) indicates a link between these two systems. The octadecanoid pathway is involved in induction of biosynthesis and release of volatiles in response to insect herbivore feeding (*5*, *6*).

We collected oral secretions (about 5 μl per caterpillar) by squeezing third to fifth instar BAW caterpillars that had been fed on corn seedlings, causing them to regurgitate (*1*). Biological activity was determined by collection and capillary gas chromatographic (GC) analysis (*7*) of volatiles from corn seedlings treated with oral secretion or subsequent fractions thereof (*8*).

The crude oral secretion was acidified, centrifuged, and filtered to remove proteins and solids (*9*). When the filtered oral secretion was fractionated on a reversed phase solid-phase extraction cartridge (*9*), the total activity of the original crude secretion eluted from the reversed phase cartridge with 50% CH_3CN in H_2O, indicating a molecule of medium polarity.

We further purified the active material from solid-phase extraction by a series of reversed-phase high-performance liquid chromatography (rpHPLC) fractionations using three sets of conditions (*10*). Only one active component, detected by monitoring ultraviolet (UV) absorption at 200 nm and with no absorption above 220 nm, eluted from the final column, and its biological activity was equivalent to that of the original crude oral secretion. The active material was extracted into CH_2Cl_2 from an acidified (pH 3) aqueous solution but not from an aqueous solution at pH 8 (*11*). All biological activity could be extracted from the organic phase back into pH 8 buffer, indicating lipid character and an acidic functional group. A CH_2Cl_2 solution of the active material was also fractionated on a

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normal-phase diol column (*12*). The active component eluted from this column with MeOH (Me designates methyl). Rechromatography of the active component on the final rpHPLC column indicated it was greater than 99% pure.

Purified active compound was applied to artificially damaged leaves of intact corn seedlings (*2*). The volatiles released were the same as those induced by treatment with BAW oral secretion. Application of only buffer resulted in the release of significantly smaller quantities and different proportions of volatiles (Fig. 2).

The active compound was identified by mass and infrared spectroscopy and by chemical transformations. Fast atom bombardment mass spectrometry (FAB-MS) analysis (*13*) indicated the presence of only one compound with diagnostic peaks at mass-to-charge ratio (m/z) 423.280 $(M + H)^+$ in the positive ion mode and at m/z 421.2733 $(M - H)^-$ in the negative ion mode. The addition of sodium chloride to the FAB matrix resulted in reduced intensity of the m/z 423.280 and the appearance of m/z 445.2628 $(MH + Na)^+$. Thus, the active compound is a weak acid with a molecular weight of 422.274 daltons for the neutral molecule in acid form, and its elemental composition is $C_{23}H_{38}N_2O_5$ (422.278 daltons).

Daughter ions of the sodium salt, m/z 445, obtained by FAB-MS/MS (*13*), included a dominant ion at m/z 427 (445 − 18), whereas daughter ions of m/z 423 gave a strong peak at m/z 405 (423 − 18), both indicating a loss of H_2O. The lower mass region showed a characteristic pattern of peaks at m/z 147, 130, 129, 101, 84, 67, and 56, which is most con-

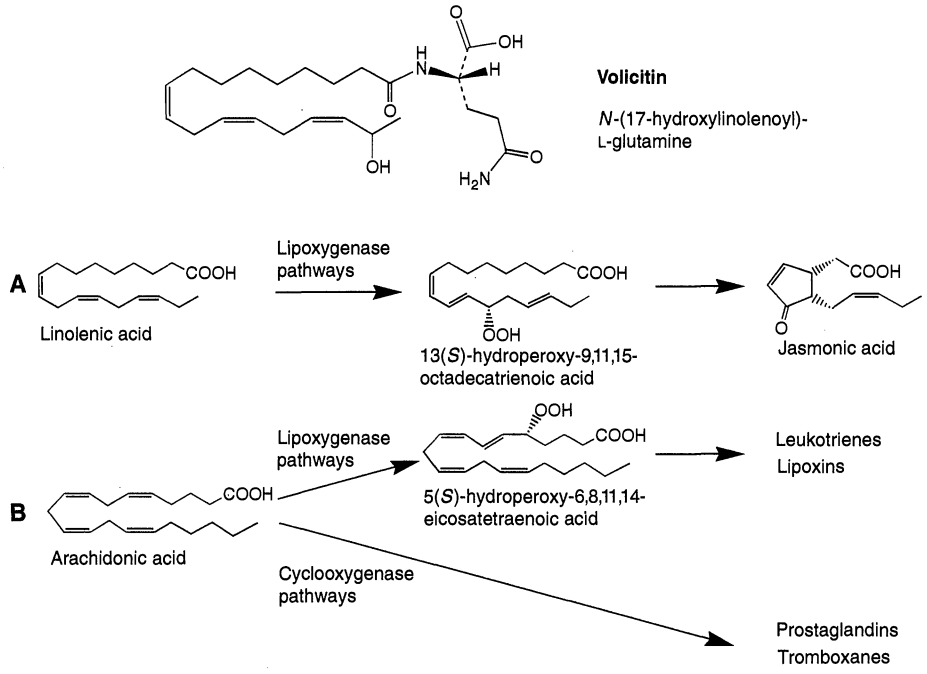
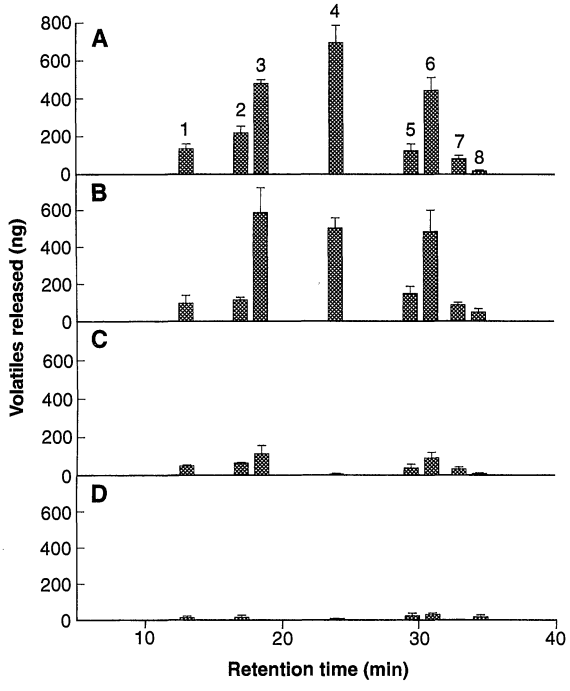


Fig. 1. Structure of volicitin. (**A**) The biosynthetic pathway leading to jasmonic acid in plants and (**B**) the biosynthetic pathways leading to prostaglandins and leukotrienes in animals.



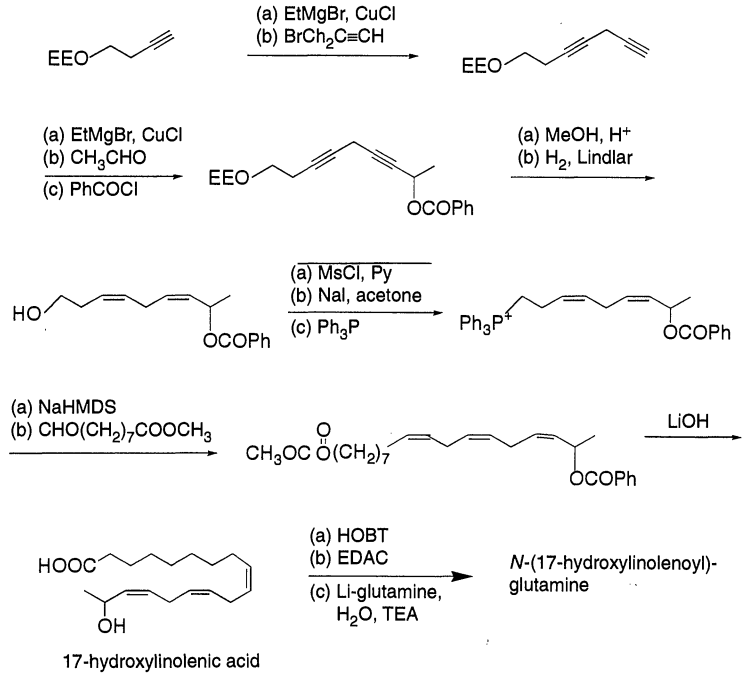


Fig. 2 (**left**). Average amount (nanograms per 2 hours) (n = 4) of volatiles collected from three intact Ioana corn seedlings that had been artificially damaged and treated with (**A**) 15 μl of BAW oral secretion per seedling on the damage sites, (**B**) 15 μl of oral secretion equivalents of pure natural volicitin, (**C**) 15 μl of buffer (*8*), or (**D**) undamaged control plants. At 9:00 p.m. a 1-cm² area of the second leaf of three-leaf seedlings was scratched with a clean razor blade and the test solution immediately rubbed over the damaged site. The next morning at 9:00 a.m. the seedlings were cut off above the root, and volatiles were collected and analyzed as described (*7, 8*). Bars with the same retention time in each graph represent the following compounds: 1, hexenyl acetate; 2, linalool; 3, (*E*)-4,8-dimethyl-1,3,7-nonatriene; 4, indole; 5, α-*trans*-bergamotene; 6, (*E*)-β-farnesene; 7, (*E*)-nerolidol; 8, (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. **Fig. 3** (**right**). Synthesis scheme for volicitin.

sistent with the electron impact (EI) mass spectrum of glutamine (*14*). Subtraction of glutamine, linked by an ester or amide bond, gave $C_{18}H_{30}O_3$ as the elemental composition of the second part of the molecule, which is consistent with a hydroxy C_{18} acid with three double bonds.

When the active sample was lyophilized and treated with MeOH and acetic anhydride (*15*), GC/MS analysis (*16*) of the product revealed two prominent peaks. Chemical ionization (CI)/MS analysis of the first of these peaks with a retention time of 21.05 min revealed a prominent $(M + 1)^+$ ion at *m/z* 144, and EI/MS analysis revealed a molecular ion at *m/z* 143 and diagnostic ions at *m/z* 84 (base peak), 56, and 41, identifying it as the methyl ester of pyroglutamate, which confirmed the presence of glutamine. The CI mass spectrum of the second peak (retention time of 27.55 min) contained a very weak *m/z* 309 $(M + 1)^+$ ion and a predominant ion at *m/z* 291 due to loss of H_2O $(M + 1 - 18)^+$. Loss of MeOH gave an ion at *m/z* 277 $(M + 1 - 32)^+$, and the loss of both H_2O and MeOH gave an ion at *m/z* 259 $(M + 1 - 18 - 32)^+$. The EI spectrum of the same peak showed no molecular ion but a strong peak at *m/z* 290 due to the loss of H_2O $(M - 18)^+$, and a fragmentation pattern of ions characteristic of a straight-chain unsaturated hydrocarbon. These results were consistent with the methyl ester of a hydroxy acid. A smaller peak in the chromatogram had retention characteristics (retention time of 26.09 min) and a mass spectrum consistent with the acetate of the same hydroxy acid methyl ester (*17*).

Fourier transform infrared analysis (*18*) of the hydroxy acid methyl ester peak from GC produced a spectrum with a weak absorption at 3646 cm^{-1}, indicating an alcohol, and absorption bands at 3019, 2935, and 2865 cm^{-1}, typical of an unbranched, nonconjugated unsaturated hydrocarbon chain. The absence of absorption bands in the 2000 to 2500 cm^{-1} and the 960 to 980 cm^{-1} regions eliminated the possibility of an acetylene or trans double bond, respectively. The intensity of the 3019 cm^{-1} peak indicated three cis double bonds. Absorption at 1758 cm^{-1} confirmed the presence of a methyl ester.

Partial reduction (*19*) of the methyl ester of the C_{18} hydroxy acid resulted in a mixture of mono- and di-unsaturated products as identified by GC/MS. Subsequent ozonolysis (*20*) of this mixture and GC/MS analysis produced three diagnostic GC peaks with $(M + 1)^+$ ions at *m/z* 187, 229, and 271, corresponding to $H(CO)(CH_2)_n(CO)OCH_3$ with $n = 7$, 10, and 13, respectively. Methyl linolenate treated in the same way gave identical products. Thus, the olefinic bonds in the chain are located on carbons 9, 12, and 15, and the alcohol group is on either the 17th or 18th carbon.

The methyl ester was saturated by treatment with PdO/H_2 overnight (*21*). GC/MS (EI) analysis of the product showed an *m/z* 299 $(M - 15)^+$ ion and *m/z* 270/271 $(M - 44/M - 43)^+$ ions, indicative of β-cleavage of a secondary alcohol on C17. EI mass spectra of a pyrrolidide derivative (*21*) of the saturated product produced diagnostic ions at *m/z* 309 $(M - 44)^+$ and *m/z* 338 $(M - 15)^+$, confirming the C17 location of the hydroxyl group.

Only an amide bond between glutamine and the acid moiety of the hydroxy acid would be consistent with the results of all analyses. Thus, the active compound isolated from the oral secretion of BAW larvae is *N*-(17-hydroxylinolenoyl) glutamine, which we name volicitin.

Racemic 17-hydroxylinolenic acid was synthesized (Fig. 3) (*22*) and coupled with D- and L-glutamine (*23*). The crude synthetic conjugates purified on HPLC showed retention characteristics identical to the natural product. FAB-MS/MS and GC/MS analyses showed the synthetic and natural products to be identical.

Volicitin synthesized with D- or L-glutamine contained about 15% of the opposing form (*24*), indicating racemization during the step in which the fatty acid was coupled to glutamine. However, the active natural compound consisted exclusively of the L-glutamine form. Enantiomerically pure D- and L-glutamine forms of synthetic volicitin were collected from a chiral column (*24*) for bioassay.

The concentration of natural volicitin was estimated by HPLC detector response to be about 20 pmol per microliter of BAW oral secretion. Bioactivity of the L-glutamine form of the synthetic volicitin was equivalent to that of oral secretion (Fig. 4). The D-glutamine form was not active (Fig. 4). Racemic 17-hydroxylinolenic acid, D-glutamine, and L- glutamine at 300 and 900 pmol per excised corn seedling showed no activity. Thus, the biologically active compound is *N*-(17-hydroxylinolenoyl)-L-glutamine. At this time the configuration about the asymmetric 17th carbon in the fatty acid chain remains unknown.

In plants, the synthesis and release of volatile compounds appear to be induced by jasmonic acid, which is produced from linolenic acid by the octadecanoid signaling pathway (*6*). Jasmonates also stimulate other physiological and defensive processes in plants (*4, 6, 25*), and the amino acid conjugates of jasmonic acid are involved in physiological and developmental processes in many plants (*6, 26*). Therefore, the presence of an elicitor that is an octadecatrienoate conjugated to an amino acid suggests that the elicitor molecule is involved with the octadecanoid pathway in the herbivore-damaged plants.

This elicitor activity is not diet-related and thus does not originate from the plants (*1*), although the fatty acid moiety is probably derived from linolenic acid obtained from the diet. The oral secretion of insects fed an artificial diet or filter paper is as active as that from insects fed on plants (*1*). Volicitin is also related in structure to eicosapentaenoic and arachidonic acids from the fungus *Phytophthora infestans* that elicit the production of fungitoxic sesquiterpenes in potato (*27*).

The octadecanoid signaling pathway in plants is similar in many ways to the eicosanoid pathways in animals that produce prostaglandins and leukotrienes (*4, 25*). In insects eicosanoids may mediate cellular responses to bacterial infections as well as regulate other physiological functions (*3*).

Both corn and cotton respond to BAW damage and to the oral secretions of BAW applied to damaged leaves by producing and releasing terpenoids and indole (*1, 2, 28–30*). Although some compounds, such as indole, ocimene, and farnesene, are released by both plants, others are unique to each plant. Both plants respond systemically to BAW oral secretion by releasing induced volatiles from undamaged leaves of injured plants (*29, 31*). In cotton the induced volatile compounds are

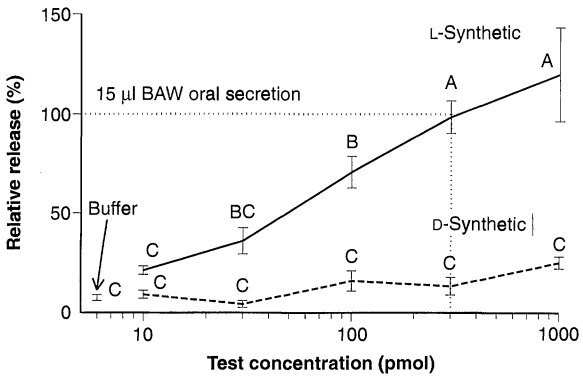


Fig. 4. Relative release of volatiles collected for 2 hours from three LG11 corn seedlings that had been treated with 10, 30, 100, 300, or 1000 pmol per plant of the D-glutamine (D-Synthetic) or the L-glutamine (L-Synthetic) forms of volicitin in 500 µl of buffer (*8*), or with buffer only. The combined amount in nanograms of caryophyllene, α-*trans*-bergamotene, (*E*)-β-farnesene, (*E*)-nerolidol, and (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene was used to calculate the release relative to that of seedlings treated with 15 µl of BAW oral secretion, which is equivalent to ~300 pmol of natural volicitin. The error bars represent standard error ($n = 5$). Data points topped by the same letter do not differ significantly [Tukey test, $P \leq 0.05$ (*33*)].

known to be synthesized de novo (*30*).

Volicitin accounts for the total activity of BAW oral secretion, and boiling the secretion for 30 min did not diminish its activity; thus, there is no evidence for enzymatic activity in eliciting volatiles in this case. In contrast, a β-glucosidase in the saliva of *Pieris brassicae* caterpillars elicits the release of volatile compounds from cabbage leaves (*32*). The sequence of events between the introduction of β-glucosidase and the emission of volatiles is unknown (*5*), but the simple release of a terpenoid or other volatile compound from a glycoside by the direct action of such an enzyme from the attacking herbivore obviously occurs by a different mechanism than the induction of de novo biosynthesis by a small molecule like volicitin. Also, it cannot account for the delayed release of herbivore-induced volatiles (*28*) or the systemic release of induced volatiles (*29*, *31*). Thus, different plant species may use different mechanisms to produce and release volatiles and may respond to different elicitors. Whether closely related insect species, particularly those that feed on the same types of host plants, produce volatile elicitors with the same or very similar structures remains unknown.

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8. Corn, *Z. mays* L., var. Ioana and LG11 sweet corn, was grown in a greenhouse in a potting soil mixture. Natural light was supplemented with 400-W high-pressure sodium lamps placed 1 m above the trays to maintain a 14-hour light (6 a.m. to 8 p.m.):10-hour dark cycle. Temperature varied from 30° to 35°C during the day and was kept at 20°C at night. BAW oral secretion (45 μl or the equivalent of each sample) was concentrated to dryness and dissolved in 1500 μl of 50 mM Na_2HPO_4 buffer, titrated to pH 8 with 1 M citric acid, which was divided into three 500-μl portions in 1-ml glass vials. At 9 to 11 p.m., a 10- to 14-day-old corn seedling was cut off above the root with a razor blade and transferred to a vial and allowed to draw up the solution for 12 hours in complete darkness. The three seedlings for each treatment were then combined in a volatile collection chamber [15 cm long, 3 cm inner diameter (ID)] under artificial light. Purified, humidified air was drawn for 2 hours through the chamber at 300 ml/min and through a polymer adsorbent (Super Q 80/100 catalog number 2735, Alltech Associates, Deerfield, IL). The adsorbent was removed from the chamber and extracted with 150 μl of CH_2Cl_2 (GC^2, Burdick and Jackson, Muskegon, MI), then internal standard (600 ng of nonyl acetate in 30 μl of CH_2Cl_2) was added.
9. Crude oral secretion was centrifuged at 16,000*g* for 30 min to eliminate solid material, and the supernatant was then filtered through a 0.22-μm sterilizing membrane (Millex GV, Millipore, Bedford, MA). An equal amount of 50 mM pH 3.3 phosphate buffer was added, and the precipitated proteins were removed by centrifugation as before. A 0.5-ml sample of the supernatant was put on a 6-ml activated, octadecyl solid-phase extraction cartridge (Bakerbond, J. T. Baker, Phillipsburg, NJ) and eluted with 2-ml volumes of H_2O, 50% CH_3CN (Low-UV HPLC grade, Burdick and Jackson) in H_2O, and CH_3CN. All fractions were concentrated to near dryness under vacuum (Speed Vac rotary concentrator, Savant Instruments, Farmingdale, NY) and redissolved in 0.5 ml of 50 mM pH 8 buffer, for bioassay.
10. The active material from solid-phase extraction was fractionated by HPLC [LDC 4100 pump with SM5000 diode array UV detector (LDC Analytical, Riviera Beach, FL)], monitoring wavelengths from 190 to 360 nm. A reversed-phase column (Waters Nova Pac C_{18} 4 μm, 4-mm ID by 150-mm long column, Waters, Milford, MA) was eluted (1 ml/min) with a solvent gradient of 0 to 25% CH_3CN in H_2O in 15 min, followed by an increase to 100% CH_3CN in 15 min. Eluate was collected in 2-ml fractions. All activity was in the 6- to 8-ml fraction, which was further fractionated on a C_{18} reversed-phase column with different selectivity (ODS-AQ S-5 200 Å, 250 mm long, 4.6-mm ID, YMC Company, Kyoto, Japan) with the same solvent gradient. All active material eluted in the 20- to 22-ml fraction. This fraction contained two overlapping peaks, which could be separated on the same column with a solvent gradient of 20 to 60% CH_3CN in H_2O from 0 to 20 min.
11. A 2-ml sample of the active fraction from the final HPLC reversed-phase column (*10*) was concentrated to dryness under vacuum and redissolved in 2 ml of H_2O, and then 100 μl of acetic acid and 2 ml of CH_2Cl_2 were added and the mixture shaken for 5 min. The H_2O and organic phases were separated and concentrated to dryness under vacuum. Bioassay of the fractions redissolved in pH 8 buffer showed all active material to be present in the organic phase, and HPLC analysis (*10*) showed the peak to be present in this fraction.
12. A 0.5-ml sample of the CH_2Cl_2 fraction was put on an activated 3-ml 10SPE diol cartridge (Bakerbond, J. T. Baker), which was eluted with 3 ml of CH_2Cl_2 and then 2 ml of MeOH. The two fractions were concentrated to dryness under vacuum and each redissolved in 0.5 ml of 50 mM pH 8 buffer for bioassay and HPLC analysis.
13. Pure elicitor [40 ng in 4 μl of 50% CH_3CN in H_2O and 1 μl of trifluoroacetic acid (TFA)] was added to a glycerol matrix and analyzed with FAB-MS on a VG Zabspec instrument (VG Analytical, Fison Instruments, Manchester, England). To obtain sodium adducts, we replaced the TFA with 1 μl of a 1 M sodium chloride solution. High-resolution mass measurements were obtained by adding polyethylene glycol (1 μl) with an average mass of 400 daltons (PEG 400) to the glycerol matrix to give reference ions of known mass for calibration of the mass scale. Possible elemental compositions were established allowing a maximum of 30 carbons, 2 to 8 oxygens, and 2, 4, or 6 nitrogens. The mass window for the calculations was limited to an error of 10 milli mass units. Daughter ion spectra were obtained from samples in the same FAB matrix as above with a tandem four-sector mass spectrometer (JEOL HX/HX110A, Tokyo, Japan). The nitrogen collision gas was adjusted to give a 60% reduction in intensity of the mother ion.
14. National Institute of Standards and Technology (Gaithersburg, MD), mass spectral library on CD-ROM, version 1.0, January 1995. Characteristic ions for glutamine are *m/z* 130, 129, 101, 84 (base peak), and 56.
15. Purified active compound equivalent to 100 μl of oral secretion was methanolyzed with MeOH and Ac_2O following the procedures of J. M. L. Mee [*Biomed. Mass Spectrom.* **4**, 178 (1977)]. The sample was concentrated to dryness, dissolved in a mixture of 50 μl of dry MeOH and 50 μl of Ac_2O, and heated to 100°C for 10 min under N_2 in a sealed ampoule. It was concentrated to dryness by a stream of N_2, then 50 μl of CH_2Cl_2 was added and the sample was analyzed by GC/MS.
16. Methanolysis products were analyzed on a Finnigan TSQ 700 mass spectrometer (Finnigan MAT, San Jose, CA) interfaced to an HP 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA). Injections were made in the splitless mode at 225°C (1 μl containing the equivalent of 2 μl of oral secretion). A polar capillary column (OV351, 25 m long, 0.25-mm ID, Scandinaviska Genetec, Kungsbacka, Sweden) was held at 60°C for 3 min and then increased 10°C per minute to 250°C and held at that temperature for 18 min. The carrier gas was He and the reaction gas was CH_4 for chemical ionization. The ion source temperature was 200°C in both EI and CI mode.
17. The acetate had a shorter retention time (26.06 min) on the polar OV-351 column than the hydroxy acid methyl ester (27.55 min), but a longer retention time (25.54 min) than the hydroxy acid methyl ester (25.38 min) on a nonpolar column (DB1, 25 m long, 0.25-mm ID, J&W Scientific, Folsom, CA). The CI mass spectrum showed ions at *m/z* 351 (weak) (M + 1), *m/z* 309 (weak) (loss of MeOH), *m/z* 291 (base peak) (loss of acetic acid), and *m/z* 259 (loss of both MeOH and acetic acid).
18. The methanolysis products were analyzed on a Hewlett-Packard (Palo Alto, CA) model 5965B Fourier transform infrared spectrometer interfaced to a model 5890 GC, equipped with a DB1 column (25 m long, 0.32-mm ID, J&W Scientific). Injections (2 μl, equivalent of 4 μl of oral secretion) were made in the splitless mode at 225°C. The carrier gas was N_2 and the temperature program the same as in (*16*).
19. The methyl ester of the hydroxy acid was partially saturated following the procedures of A. B. Attygalle, G. N. Jham, A. Svatos, R. T. S. Frighetto, and J. Meinwald [*Tetrahedron Lett.* **36**, 5471 (1995)]. After concentration to dryness the sample was redissolved in 10 μl of ethanol, then 30 μl of 10% hydrazine in ethanol and 30 μl of 0.6% hydrogen peroxide in ethanol was added and the solution heated to 60°C for 1 hour. After the solution cooled to room temperature, 35 μl of 5% HCl in H_2O was added. The solution was extracted with two 40-μl portions of hexane (GC^2, Burdick and Jackson) and the hexane solution washed with four 50-μl portions of H_2O before being analyzed by GC/MS (*24*).
20. Partially reduced hydroxy acid methyl ester (*19*) was ozonized following the procedures of M. Beroza and B. Bierl [*Anal. Chem.* **38**, 1976 (1966); *ibid.* **39**, 1131 (1967)] and the product analyzed by GC/MS (*16*).
21. The hydroxy acid methyl ester was dissolved in 50 μl of ethyl acetate, a few grains of PdO was added, and saturation was achieved by bubbling H_2 through the solution for 18 hours. A pyrrolidide derivative of the saturated methyl ester was prepared following the procedures of B. Å. Andersson [*Prog. Chem. Fats Other Lipids* **16**, 279 (1978)].
22. Racemic 17-hydroxy linolenic acid was synthesized (Fig. 3) by the condensation of the appropriate triphenylphosphonium ylid, prepared from the alkyl triphenylphosphonium iodide by the silazide method [H. J. Bestman, W. Stransky, O. Vostrowsky, *Chem. Ber.* **109**, 1694 (1976); H. J. Bestman *et al.*, *Liebigs Ann. Chem.* **1987** 417 (1987)], with methyl 9-oxononanoate [F. C. Pennington, W. D. Clemer, W. M. McLamore, V. V. Bogert, I. A. Solomons, *J. Am. Chem. Soc.* **75**, 109 (1953)]. The phosphonium salt was obtained by standard methodology from the ethoxy ethyl ether of 3,6-heptadiyn-1-ol [W. Huang, S. P. Pulaski, G. Meinwald, *J. Org. Chem.* **48**, 2270 (1983)] by sequential condensation with acetaldehyde and benzoyl chloride, followed by deprotection, partial hydrogenation, and conversion of the primary alcohol to the phosphonium iodide.
23. D- or L-glutamine (1.1 to 1.2 equivalents) was converted into its lithium salt by the procedures of R. Kramell, J. Schmidt, G. Schneider, G. Sembdner, and K. Schreiber [*Tetrahedron* **44,** 5791 (1988)] and coupled to the hydroxy acid by procedures modified from J. C. Sheehan,

J. Preston, and P. A. Cruickshank [*J. Am. Chem. Soc.* **87**, 2492 (1965); W. Konig and R. Geiger, *Chem. Ber.* **103**, 788 (1970)]. The coupling was carried out in *N,N*-dimethylformamide (DMF) (Sigma) with addition of 5 to 10% H_2O to increase solubility of the salt and 1.2 equivalents of 1-hydroxybenzotriazole (Sigma) to reduce racemization of glutamine. The product was purified by rpHPLC (*10*) on the YMC column eluted with 25% CH_3CN in 0.4 mM ammonium acetate buffer (Aldrich) at a flow rate of 1.2 ml/min. The retention time of the synthetic and natural elicitor was identical.

24. The D- and L-glutamine forms of synthetic volicitin were separated on a 250-mm, 4.6-mm ID chirobiotic T column (Advanced Separation Technologies, Whippany, NJ) eluted with 10% CH_3CN in 10 mM ammonium acetate buffer, pH 4.5, at a flow rate of 1 ml/min (*10*). The synthetic L-form had a retention time of 5.25 min, identical to that of the natural elicitor, and the D-form had a retention time of 9.03 min.
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33. Significant differences in relative release rates of volatiles were tested by Tukey's studentized range test after analysis of variance with a significance level of 5% (SYSTAT, Systat Inc., Evanston, IL).
34. This project was funded in part by a grant from the Swedish Natural Science Research Council. We thank H. Karlsson, A. T. Proveaux, and D. Powell for assistance with mass spectrometric analysis, J. Lockerman and S. Sharp for oral secretion collection, and M. Brennan for technical assistance. We also thank J. G. Millar, C. A. Ryan, and G. G. Still for helpful comments on the manuscript.

24 January 1997; accepted 17 March 1997

A Tonic Hyperpolarization Underlying Contrast Adaptation in Cat Visual Cortex

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The firing rate responses of neurons in the primary visual cortex grow with stimulus contrast, the variation in the luminance of an image relative to the mean luminance. These responses, however, are reduced after a cell is exposed for prolonged periods to high-contrast visual stimuli. This phenomenon, known as contrast adaptation, occurs in the cortex and is not present at earlier stages of visual processing. To investigate the cellular mechanisms underlying cortical adaptation, intracellular recordings were performed in the visual cortex of cats, and the effects of prolonged visual stimulation were studied. Surprisingly, contrast adaptation barely affected the stimulus-driven modulations in the membrane potential of cortical cells. Moreover, it did not produce sizable changes in membrane resistance. The major effect of adaptation, evident both in the presence and in the absence of a visual stimulus, was a tonic hyperpolarization. Adaptation affects a class of synaptic inputs, most likely excitatory in nature, that exert a tonic influence on cortical cells.

Adaptation is a fundamental sensory process that allows neurons to respond with high sensitivity to a wide range of sensory inputs. One aspect of sensory stimuli to which neurons in the visual cortex adapt is contrast (*1–4*): Adaptation to high contrasts increases the threshold contrast required to evoke a given response, whereas adaptation to low contrasts decreases that threshold (*3*). Adaptation therefore acts as a gain control mechanism that maximizes the sensitivity of cortical cells to the average contrast of their most recent stimuli (*4, 5*). Recent theories suggest that adaptation may have an even broader role in enhancing the coding efficiency of the cerebral cortex (*6*).

Previous studies of contrast adaptation in the visual cortex were based on extracellular measurements of spike responses. Although these measurements indicate what a cell communicates to the rest of the brain, they do not provide many clues to the cellular mechanisms underlying adaptation. To investigate these mechanisms, we made intracellular recordings from 27 neurons of the cat primary visual cortex (*7*).

Intracellular records for a cortical simple cell are shown in Fig. 1. In our sample of 15 simple cells, the spike responses to drifting gratings and their dependence on contrast were consistent with those observed in extracellular experiments: (i) The cells' firing rates (Fig. 1A) were strongly modulated by the passage of each cycle of the stimulus grating (*8*). (ii) The amplitude of this modulation, as measured by the first harmonic (F1) component of the response at the stimulus temporal frequency, grew with contrast. (iii) In all cells, the relation between stimulus contrast and the F1 component of the firing rate (Fig. 1E, solid circles) was well fit by a hyperbolic ratio (*9*),

$$R(c) = R_{max}c^n/(\sigma^n + c^n) \qquad (1)$$

where c is stimulus contrast, R is the cell's response, and R_{max}, σ, and n are free parameters.

The fluctuations in membrane potential evoked by drifting gratings were nearly sinusoidal at all contrasts (Fig. 1C, thick traces). Increasing contrast had a dual effect on the responses: It increased the amplitude of the stimulus-modulated (F1) component of the response, and it increased the mean level around which the modulation occurred (the DC component) (*10*). The dependence of both the F1 and DC components on contrast was also well fit by a hyperbolic ratio, except for a decrease at the highest contrasts observed in most of our cells (Fig. 1, D and F, solid circles).

The responses described so far were obtained while the cell was adapted to low contrasts, that is, while the seven test stimuli (1, 2, 4, 8, 16, 32, and 64% contrast) were interleaved with exposure to 1.5% contrast adapting stimuli. To change the cell's adaptation state, we alternated these low-adaptation measurements of contrast response with high-adaptation measurements in which the same seven test stimuli were interleaved with exposure to 47% contrast adapting stimuli (*3, 11*). Consistent with previous reports (*1–4*), adaptation reduced the spike responses (Fig. 1B). In particular, it shifted the contrast response curve to the right (*3, 4*) (Fig. 1E, open circles); adaptation thus decreased the sensitivity of the cell by half, so that obtaining a given response amplitude required twice the stimulus contrast.

The effect of adaptation on the membrane potential responses can be seen by comparing the thick and thin traces (1.5% and 47% contrast, respectively) in Fig. 1C. Surprisingly, the changes observed in the F1 component of the spike responses were not mirrored by changes in the size of the sinusoidal membrane potential modulation. Indeed, there was neither a rightward nor a downward shift in the contrast response curve derived from the F1 component of the membrane potential (Fig. 1D). The main effect of adaptation on the membrane potential was to shift the membrane potential down by as much as 15 mV (Fig. 1C, thin traces). This hyperpolarization was reflected in a downward shift of the contrast response curve constructed from the DC component of the membrane potential (Fig. 1F, open circles). Because of the lack of adaptation effects on the F1 component of the membrane potential, this downward shift

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