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N-(17-Acyloxy-acyl)-glutamines: Novel Surfactants from Oral Secretions of Lepidopteran Larvae†

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N-(17-Acyloxy-acyl)-glutamine conjugates such as *N*-(17-linolenoyloxy-linolenoyl)-glutamine (**6**), *N*-(17-linolenoyloxy-linoleoyl)-glutamine (**7**), *N*-(17-linoleoyloxy-linolenoyl)-glutamine (**8**), and *N*-(17-linoleoyloxy-linoleoyl)-glutamine (**9**) were identified as novel surfactants in the oral secretion of several lepidopteran larvae (*S. exigua*, *S. littoralis*, *S. frugiperda*, and *H. virescens*) by LC-MS/MS and chemical degradation. Authentic reference compounds were synthesized via a dissymmetric bis-Wittig approach and confirmed the assigned structures.

Introduction

Conjugates of fatty acids with amino acids, in particular glutamine and glutamate, are common constituents of gut secretions of lepidopteran larvae.[1−3] The compounds are also found in the digestive tract of other arthropods, even in spiders, crabs, and crickets.[4] Another rich source of *N*-acyl-amino acids is microorganisms that produce a large variety of antibacterial or antifungal surface-active agents. Some enhance the bioavailability of surfaces for growth, others regulate the attachment and/or detachment of microorganisms to surfaces (*quorum sensing*).[5] Besides simple *N*-acyl-amino acids, there exist more complex structures mimicking phospholipids of membranes.[6] In many cases the lipid moiety of the amino acid conjugates comprises a 3-hydroxy core fatty acid acylated with a second fatty acid. The first members of such complex amphiphilic lipids were isolated from culture media of different Gram negative bacteria, among them are *Pseudomonas*, *Streptomyces*, and *Flavobacterium* species.[7] The zwitterionic, acylated amide lipids are interchangeable in function with the amphiphilic phospholipids of membranes and become major products under phosphate-limited conditions.[6] Recently, the first hydroxylated *N*-acyl glutamines were found in gut secretions of insects.

N-(17-Hydroxy-linolenoyl)-L-glutamine (volicitin, **1**) was isolated from oral secretions of lepidopteran larvae (*Spodoptora* spp.) and reported to elicit the biosynthesis of volatiles after introduction into the damaged leaf by the feeding insect.[1,3,8,9] By synthesis and comparison with the natural secretions, the chiral center in the fatty acid moiety was shown to be always 17*S* with ≥94% ee.[10] Volicitin (**1**) is, in some insects, accompanied by minor amounts of another series of oxygenated conjugates such as *N*-(15,16-epoxy-linoleoyl)-glutamine (**4**) and *N*-(15,16-dihydroxy-linoleoyl)-glutamine (**5**).[11] According to recent biosynthetic studies, the amide bond between the fatty acid and glutamine is apparently made by commensal microorganisms living in the insect gut.[12] Owing to their structural analogy to microbial lipids and owing to the involvement of bacteria in their biosynthesis, we were encouraged to investigate the oral secretions of lepidopteran larvae for the presence of *O*-acylated derivatives of volicitin (**1**) related to the previously known 3-*O*-acyl amino acids from microorganisms. Here we report on the isolation and synthesis of a novel series of *N*-(17-acyloxy-

† Dedicated to Prof. Dr. W. Steglich on the occasion of his 70th birthday.

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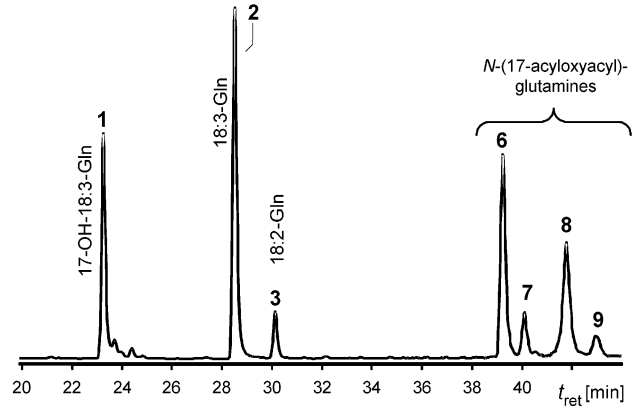
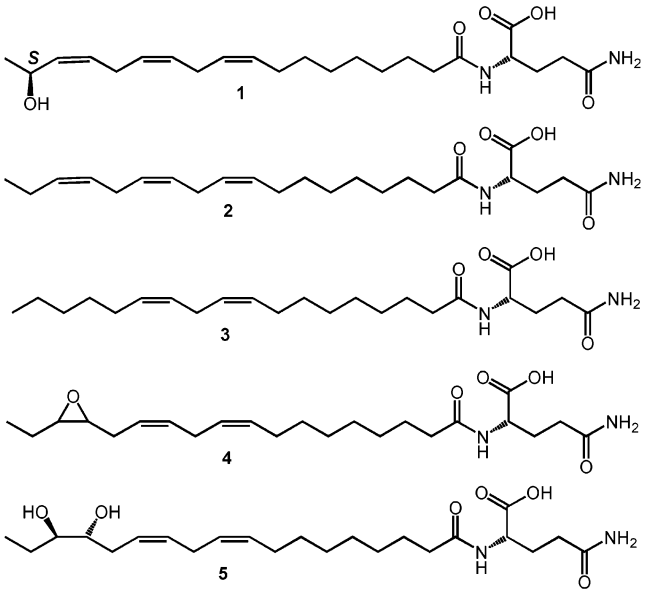


FIGURE 1. *N*-Acyl-glutamines in oral secretions from larvae of *Spodoptera exigua* (RP-18, for HPLC conditions see Experimental Section).

SCHEME 1. *N*-Acylglutamines from the Regurgitant of Spodopteran Larvae



acyl)glutamines that occur widespread in oral secretions of lepidopteran larvae.

Results and Discussion

Isolation and Structural Analysis of *N*-(17-Acyloxy-acyl)-glutamines. Oral secretions of several lepidopteran larvae were collected into glass capillaries by gently squeezing the larva with a forceps behind the head which caused immediate regurgitation. After collection, the oral secretions were investigated for higher molecular weight *O*-acyl conjugates by HPLC-MS on reversed phase (RP18), using gradient elution as shown in Figure 1. The three first eluting compounds were readily identified with reference compounds as volicitin (**1**), *N*-linolenoyl-glutamine (**2**), and *N*-linoleoyl-glutamine (**3**) (Scheme 1).[1,2] The APCI-mass spectrum of the first unknown compound **6** (40.5 min) displayed an intense quasimolecular ion at *m*/*z* 683 $[M + H]^+$ and an $[M + Na]^+$ adduct at *m*/*z* 705 (see Figure 2). Fragment ions at *m*/*z* 405, 259, 147, and 130 were identical with corresponding fragment ions of volicitin (**1**), suggesting a close structural relationship between the two compounds. The fragment at *m*/*z* 147 is common to all conjugates of this type and indicates the presence of glutamine. Since MS/MS experiments with **6** established *m*/*z* 405 to be derived from *m*/*z* 683, the difference of 278 Da suggested the presence of an additional linolenic acid molecule in the conjugate.

This assumption was confirmed by HR-MS ($C_{41}H_{67}N_2O_6$ for the quasimolecular ion at *m*/*z* 683) and by methanolysis of **6**, which released the methyl esters of linolenic acid and 17-hydroxy-linolenic acid from the conjugate. Additional compounds, varying in the degree of unsaturation of their fatty acid building blocks, were present in the oral secretions. The APCI mass spectrum of compound **7** (see Figure 1 and Scheme 2) shows a quasimolecular ion $[M + H]^+$ at *m*/*z* 685, along with a rather weak fragment at *m*/*z* 407, suggesting a 17-hydroxy-linoleic acid as the central building block. This most obvious difference between the mass spectra of **6** and **7** is due to the ease of elimination of allylic substituents from the volicitin-type conjugates, yielding terminally conjugated products with the most abundant ion at *m*/*z* 405 (100%).[12,13] Derivatives of 17-hydroxy-linoleic acid are less prone to elimination reactions and, hence, display only a weak fragment at *m*/*z* 407 (16%). Methanolysis of **7** yielded the methyl esters of the fatty acid core 17-hydroxy-linoleic acid and linolenic acid. The two other compounds were identified as *N*-(17-linoleoyloxy-linolenoyl)-glutamine (**8**) and *N*-(17-linoleoyloxy-linoleoyl)-glutamine (**9**).

Information on the positions of the double bonds in the novel conjugates was obtained by ozonolysis of purified **6** and **7**. For example, oxidative cleavage of the conjugate **7** (Scheme 3) delivered the dialdehyde ester **10** (determined by GC-MS), along with the polar amino acid conjugate **11** (determined by LC-MS), confirming the assigned structure and its origin from common unsaturated fatty acids such as linoleic and linolenic acid.

Synthesis of *N*-(17-Linolenoyloxy-linoleoyl)-glutamine (7). To prove the assigned structures and to obtain material for bioassays, the conjugate **7** was prepared following a previously established protocol for the asymmetric synthesis of volicitin (**1**) (Scheme 4).[13]

The key step of the approach is the one-pot sequential olefination of the bis-phosphorane **13**, obtained from the corresponding bis-phosphonium salt by deprotonation with $KN(SiMe_3)_2$ to achieve a high (*Z*)-selectivity.[14] The first carbonyl component, the aldehyde **12**, already carrying the linolenic acid moiety, was added at −78 °C, and the reaction mixture was allowed to come to rt within 1 h. After the mixture was recooled to −78 °C, 9-oxo-nonanoic acid (2-trimethylsilyl)-ethyl ester (**14**) was introduced as the second carbonyl component. After purification and chromatography on silica gel, the (2-trimethylsilyl)-ethyl ester of 17-linolenoyloxy-linoleic acid (**15**) was obtained in 26% overall yield. According to the ^{13}C NMR spectra, introduction of each of the two double bonds occurred with ≥90% (*Z*)-selectivity. Removal of the (2-trimethylsilyl)-ethyl ester moiety with TBAF proceeded smoothly and without affecting the linolenoyl ester moiety. Final condensation of the free acid with

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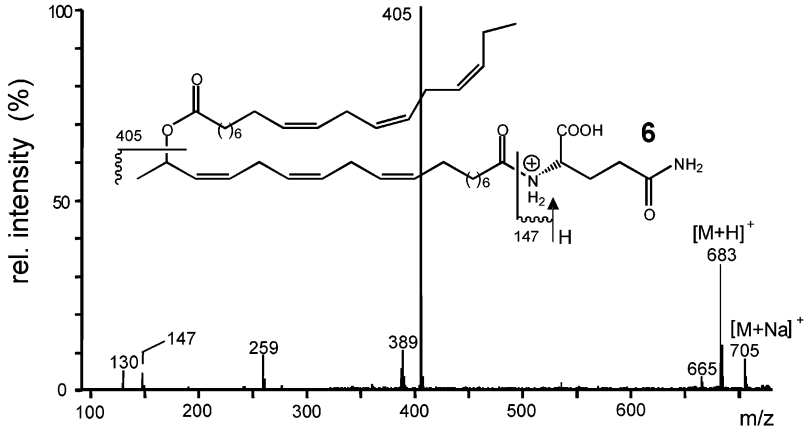
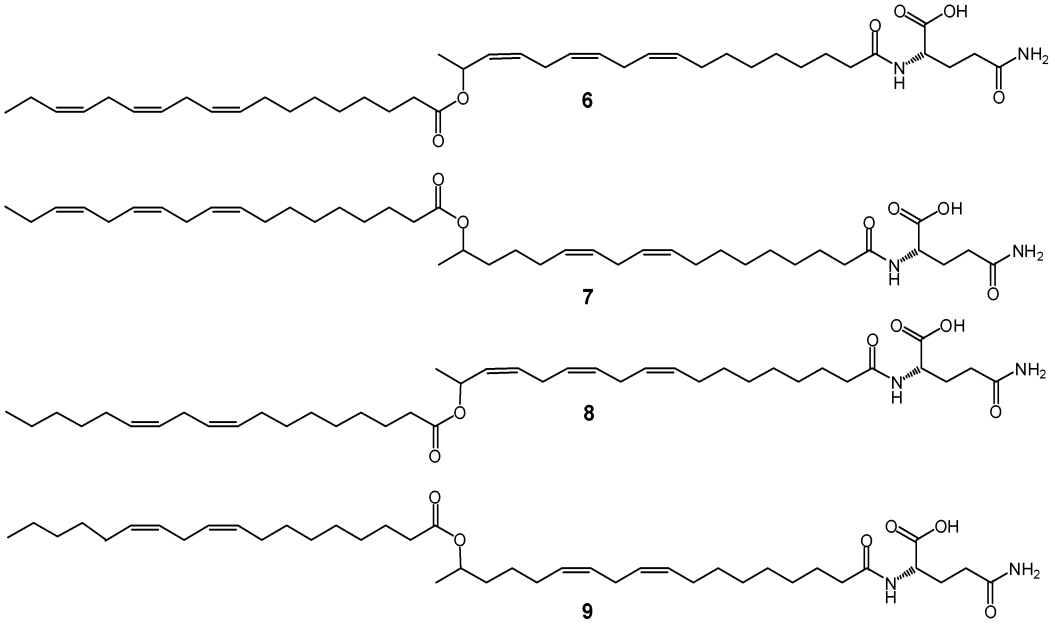
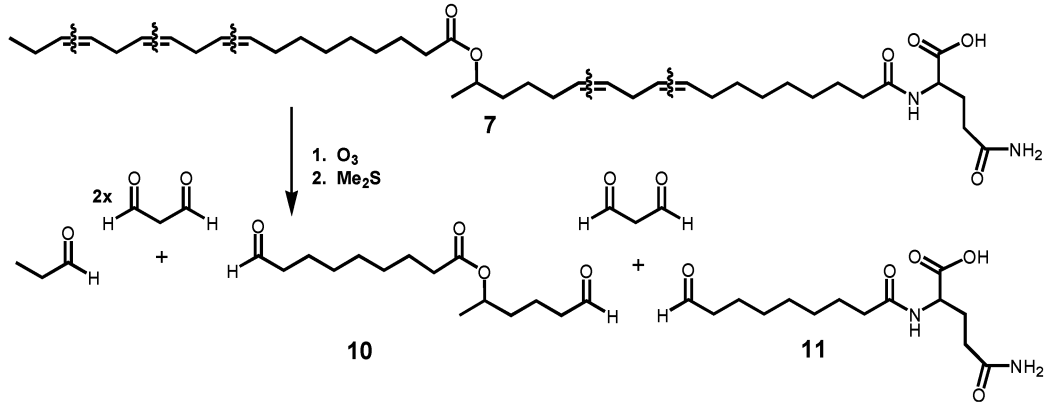


FIGURE 2. APCI-mass spectrum of *N*-(17-linolenoyloxy-linolenoyl)-glutamine **(6)**.

SCHEME 2. *N*-(17-Acyloxyacyl)-glutamines from the Regurgitant of Spodopteran Larvae



SCHEME 3. Ozonolysis of *N*-(17-Linolenoyloxy-linoleoyl)-glutamine (7)



L-glutamine was achieved as described[13] and afforded *N*-(17-linolenoyloxy-linoleoyl)-L-glutamine (**7**), identical in all respects with the natural product.

The two building blocks **12** and **14** required for the synthesis of **15** were obtained as outlined in Scheme 5. Grignard reaction of the dioxolane **17** with 2-methyloxirane yielded the alcohol **18**,[15] which was esterified with linolenic acid in the presence of *N*,*N*-(dimethylamino)-pyridine and dicyclo-hexylcarbodiimide.[16]

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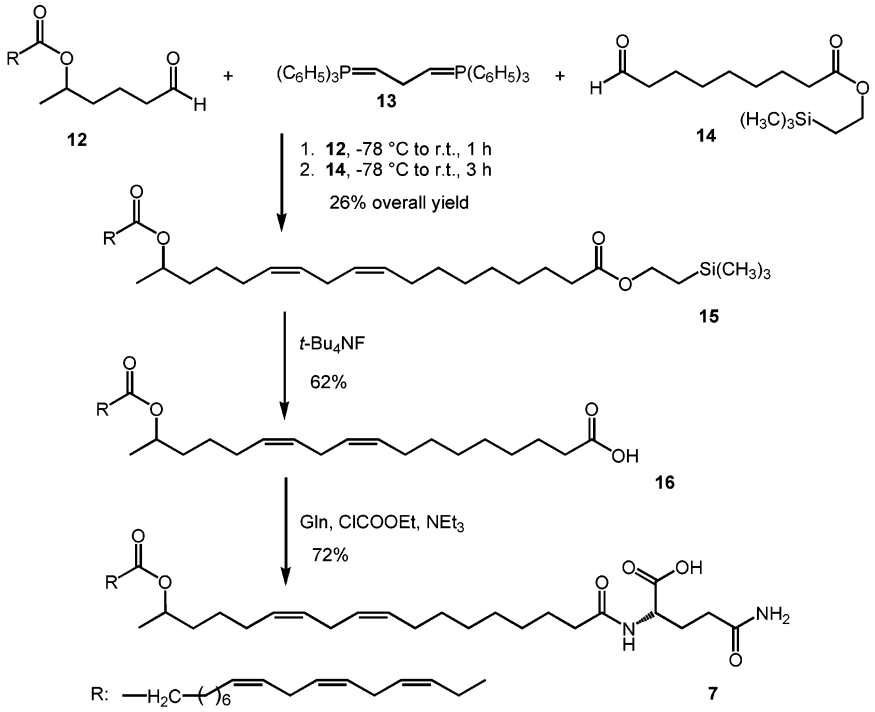
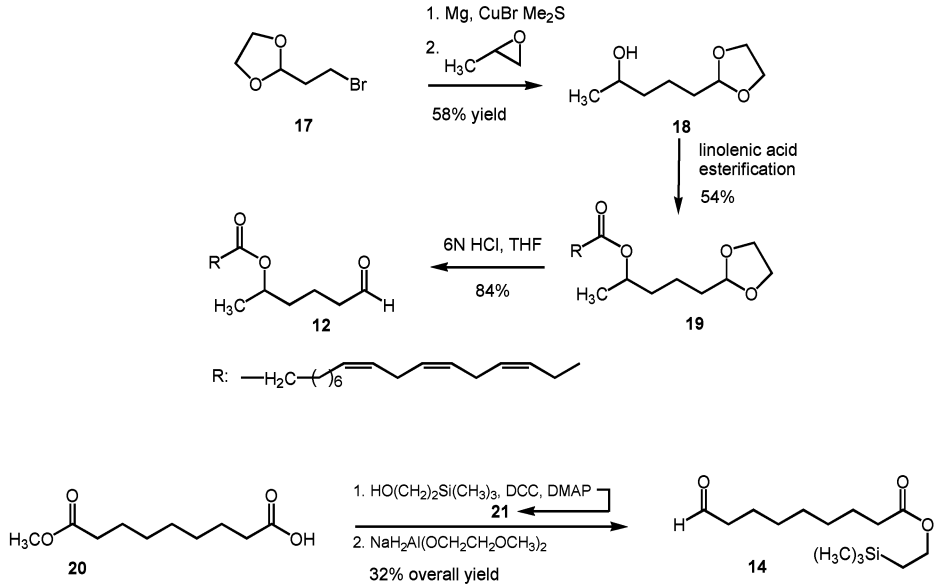
SCHEME 4. Synthesis of *N*-(17-Linolenoyloxy-linoleoyl)-L-glutamine (7)



SCHEME 5. Synthesis of the Substructural Elements of 12 and 14



Treatment of the 1,3-dioxolane **19** with aq THF/HCl selectively removed the protecting group and afforded **12** in about 30% overall yield. The 9-oxo-nonanoic acid (2-trimethylsilyl)-ethyl ester (**14**) was available from nonanedicarboxylic acid monomethyl ester (**20**) by esterification with (2-trimethylsilyl)-ethanol[17,18] and subsequent regioselective reduction of the less hindered methyl ester **21** with $NaH_2Al(OCH_2CH_2OMe)_2$.[19] **14** was obtained with 32% overall yield.

The synthetic concept (Scheme 4) has a high potential and flexibility, since it allows the use and combination of different substructural elements, tolerates a number of functional groups, and is easily extended to highly unsaturated or isotopically labeled fatty acids and related compounds.[14]

Although direct esterification of the 17-hydroxy group of volicitin (**1**) with activated derivatives of linolenic or linoleic acid would provide a more direct route to the title compounds, this approach suffered from competing elimination of the 17-hydroxy substitutent. Similar observations have been reported for derivatization of the mayolenes.[20] An attempt to acylate volicitin (**1**) with linolenic acid or its vinyl ester by enzyme catalysis (porcine

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TABLE 1. *N*-(17-Acyloxy-acyl)-glutamines in Lepidopteran Larvae[a]

insects	1	2	6	7	8	9
Spodoptera exigua	++	++	+	+(+)	+	+(+)
Spodoptora littoralis	++	++	+(+)	+	+	+
Spodoptera frugiperda	++	++	+	+	+	+
Heliothis virescens	++	++	+	+(+)	+	+

[a] Key: ++, major *N*-acyl-glutamines (>30%); +, minor compounds (1%-30%, values in parentheses cover occasional strong shifts in the composition of the regurgitant).

pancreas lipase in decane) was more successful and yielded the conjugate **6**, albeit in rather low yield (<5% yield). Optimization of this approach is under investigation.

Analysis of Oral Secretions from Selected Lepidopteran Larvae. Besides the known fatty acid amides with glutamine such as **1**, **2**, and **3**, the regurgitant from the larvae listed in Table 1 showed different amounts of the novel *N*-(17-acyloxy-acyl)-glutamines. The compounds were not observed in oral secretions of larvae that fail to produce *N*-(17-hydroxy-acyl)-glutamines [e.g., volicitin (**1**)] like the tobacco hornworm (*Manduca sexta*).

Moreover, the quantitative composition of the amino acid conjugates in the regurgitants shows remarkable variations and appears to be dependent on the rearing conditions. Occasionally, no or very low amounts of *N*-(17-acyloxy-acyl)-glutamines were found. The novel conjugates may be either produced by acylation of volicitin (**1**) or *N*-(17-hydroxylinoleoyl)-glutamine, or by acylation of a 17-hydroxy acid (linolenic or linoleic acid type) by microbial enzymes. First experiments with isolated microorganisms or even a pure enzyme isolated from a commensal gut bacterium (unpublished results) with simple aliphatic or hydroxylated fatty acids and natural amino acids for conjugation revealed a very broad substrate tolerance of the biocatalyst without a clear preference for a certain fatty acid or amino acid, suggesting that amide bond formation can occur with both types of fatty acid substrates.

Conclusions

Besides the previously known *N*-acyl glutamines, volicitin (**1**) and *N*-(17-hydroxy-linoleoyl)-glutamine, a series of new *N*-(17-acyloxy-acyl)-glutamines is found in the oral secretion of lepidopteran larvae. The *N*-(17-acyloxy-acyl)-glutamines **6**, **7**, **8**, and **9** are formally derived from volicitin (**1**) or *N*-(17-hydroxy-linoleoyl)-glutamine by additional acylation with linolenic or linoleic acid. Structurally related compounds were previously only known as microbial metabolites, and their presence in the insect gut may be considered as another piece of evidence for the impact of commensal microorganisms on the chemical composition within the gut of insects. The lipid moieties of the novel conjugates **6** to **9** exhibit similarities to the recently isolated mayolenes from glandular hairs of the lepidopteran larva *Pieris rapae*.[20] Mayolenes are composed of an 11-hydroxy-linolenic acid esterified to a series of straight chain saturated fatty acids of 14 to 20 carbon atoms, with palmitic acid and stearic acid dominating. The compounds are potent deterrents against ants and other enemies. Another example of a hydroxylated, but nonconjugated pentadienyl segment is given by the ethyl esters of 13-hydroxy-arachidonate and 13-hydroxyeisosa-5,8,11,14,17-pentaenoic acid, both of which were isolated from red calcareous algae from the genus *Lithothamnion*.[21]

N-acyl amino acids occur widespread in the digestive organs of insects and beyond (spiders, crabs, etc.).[4] They may serve the organism as surfactants to emulsify food lipids. In some cases, they may also have a defensive function, since, for example, larvae of sawflies such as *Neodiprion lecontei* discharge their enteric fluid onto attacking enemies.[22] A similar behavior is known for some Spodopteran larvae. Unlike the very unstable mayolenes, the conjugates **6**−**9** are less prone to elimination of the acyloxy substituent; even under the very strongly basic conditions (pH ca. 9) of the insect gut the compounds remain intact. The pronounced tendency of compounds such as **6** to form micelles at very low concentrations may protect the sensitive allylic acyloxy moiety against the basic milieu. The critical micellar concentration of, e.g., *N*-linolenoyl-glutamine (**2**) (cmc ca. 25 μM) is much lower than that of the commonly employed detergent sodium dodecyl sulfate (SDS; cmc 8−10 mM) and competes well with that of surfactin,[23] another powerful and well-studied bacterial amphiphile (cmc ca. 9 μM). Plant feeding larvae introduce the compounds with their regurgitant into the damaged leaves and, owing to their strong surface activity, the conjugates are able to depolarize plant cells and thereby elicit defensive programs in plants. The mode of action of the *N*-acyl amino acids on plant systems is currently evaluated and will be reported soon.

Experimental Section

General Methods. Reactions were performed under Ar. Solvents were dried according to standard methods. ^{1}H and ^{13}C NMR were recorded at 400 and 500 MHz, respectively. Chemical shifts of ^{1}H and ^{13}C NMR are given in ppm (δ) based on solvent signals: $DCCl_3$ 7.26 (^{1}H NMR) and 77.70 ppm (^{13}C NMR). For APCI-LC-MS/MS a microbore RP18-column 125 cm × 2 mm, 2 μm was used. High-resolution ESI-mass spectrometry was performed by direct injection of the purified sample. High-resolution mass spectrometry was performed on a double-focusing magnetic sector mass spectrometer (geometry EBE). For GC-MS (70 eV) a DB5 column was used (15m × 0.25 mm, 0.25 μm); helium served as carrier gas. Silica gel: Si 60 (0.200−0.063 mm) was used for chromatography.

Rearing of Lepidopteran Larvae and Collection of Oral Secretion: Artificial Diet. Ground white beans (500 g) were soaked overnight in water (1200 mL) and ascorbic acid (9 g), parabene (9 g), aq formaldehyde (4 mL, 36.5%), and agar (75 g), boiled in 1000 mL of H_2O, were added. After cooling the mixture solidified to a white waxy solid.[24]

Rearing of Insect larvae. Larvae were hatched from eggs and reared on the agar-based artificial diet. Plastic cages with the diet and the larvae were kept at 23−25 °C under a light-dark regime with 16 h of illumination.

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Collection of Oral Secretion. Regurgitant was collected from larvae by slight squeezing of the animals with tweezers and collection of the discharged enteric liquid with glass capillaries (1 mm i.d.).

Analysis of Oral Secretions. The collected regurgitant was diluted with the same volume of MeOH to precipitate proteins. After centrifugation at 13 000 U/min (16 000 g) for 5 min, the supernatant was directly used for LC-MS analysis. Separation was achieved by HPLC (RP18, 150 mm × 2 mm, 2 μm; Grom, Herrenberg, Germany) with programmed gradient elution. A typical analysis was performed with a flow rate of 0.2 mL min^{-1}, using a binary gradient of two solvent systems (A: H_2O with 0.1% AcOH; and B: MeCN with 0.1% AcOH) starting with 100% A (3 min) programmed to 100% B in 27 min maintained for 15 min prior to equilibration to the starting conditions. The relevant compounds eluted between 39 and 51 min and were investigated by MS/MS experiments (collision energy: 15−25%) and HR-ESI-MS.

***N*-(17-Linolenoyloxy-linolenoyl)-glutamine (6):** 39.5 min; APCI-MS $[M + Na]^+$ 705 (8), $[M + H]^+$ 683 (28), 405 (100), 259 (6), 147 (1); HR-ESIMS $[M + H]^+$ calcd for $C_{41}H_{67}N_2O_6$ 683.4999, found 683.4979.

***N*-(17-Linolenoyloxy-linoleoyl)-glutamine (7):** 40.5 min; APCI-MS $[M + Na]^+$ 707, $[M + H]^+$ 685 (100), 667 (5), 407 (16), 261 (2), 147 (1), 130 (1); HR-ESI-MS $[M + H]^+$ calcd for $C_{41}H_{69}N_2O_6$ 685.5156, found 685.5143.

***N*-(17-Linoleoyloxy-linolenoyl)-glutamine (8):** 41.5 min; APCI-MS $[M + Na]^+$ 707, $[M + H]^+$ 685 (27), 405 (100), 259 (5), 147 (2).

***N*-(17-Linoleoyloxy-linoleoyl)-glutamine (9):** 43.0 min; APCI-MS $[M + H]^+$ 687 (100), 407 (18), 261 (1),147 (1).

Ozonolytic Cleavage of the Conjugates 6 and 7. *N*-(17-Acyloxy-acyl)-glutamine conjugates from the regurgitant (500 μL) of larvae of *S. exigua* were purified by HPLC (see above). After removal of the solvent by a gentle stream of argon and drying under high vacuum, the residue was dissolved in dry MeOH (1 mL), and ozone (ca. 15 mg/min) was passed through the solution for 5 min (−78 °C). The ozonides were reduced by addition of Me_2S (1 μL), and after 5 min the solvent was removed by a stream of argon. Following an additional treatment with diazomethane, the polar cleavage products were identified by GC-MS. Ozonolytic degradation of conjugates **6** and **7** yielded the following fragments: 5-(9-oxo-nonanoyl)-hexanal (**10**) [GC-MS 155 (100), 115 (24), 109 (48), 99 (88), 81 (90), 69 (35), 67 (26), 55 (81), 44 (40)] and 9-oxo-nonanoic acid methyl ester [GC-MS 158 (7), 155 (18), 143 (26), 111 (39), 87 (67), 83 (45), 74 (100), 59 (40), 55 (75)]. *N*-(9-Oxo-nonanoyl)-glutamine (**11**) was identified by LC-MS. APCI-MS $[M + H]^+$ 301 (100), 283 (37), 155 (2).

Synthesis of *N*-(17-Acyloxy-acyl)-glutamines: Nonanedioic Acid (2-Trimethylsilyl)-ethyl Ester Methyl Ester (21). To a chilled and well-stirred solution of monomethyl ester **20** (2 g, 10 mmol), *N*,*N*-(dimethylamino)-pyridine (80 mg, 0.66 mmol),[16] and 2-trimethylsilylethanol[17,18] (2.1 g, 15 mmol) in dry dichloromethane (10 mL) was added, under argon, dicyclohexylcarbodiimide (2.3 g, 11 mmol) in 10 mL of the same solvent. After the mixture was stirred for 5 min at 0 °C, stirring was continued for 3 h at rt. The precipitated urea was filtered off and the mixture was extracted with 0.5 N HCl and satd $NaHCO_3$ solution. After drying (Na_2SO_4) the solvent was removed under reduced pressure. Purification was achieved by chromatography on silica gel, using petroleum ether:ethyl acetate (9:1, v:v) for elution. Colorless liquid (2.16 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.10−4.17 (m, 2H), 3.64 (s, 3H), 2.28 (t, J = 7.55 Hz, 2H), 2.25 (t, J = 7.43 Hz, 2H), 1.52−1.65 (m, 4H), 1.22−1.36 (m, 6H), 0.92−0.99 (m, 2H), 0.02 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.86, 174.58, 63.02, 52.08, 35.10, 34.68, 29.59, 29.53, 25.53, 25.51, 17.96, −0.84; IR (KBr, film) 2951, 2861, 1735, 1437, 1355, 1250, 1172, 1062, 938, 856, 838 cm^{-1}; EI-MS 302 ($M^{+\bullet}$, 0.5) 287 (3), 259 (45), 243 (13), 227 (9), 185 (19), 173 (45), 159 (24), 117 (29), 75 (49), 73 (100), 59 (12); EI-HRMS calcd for $C_{15}H_{30}O_4Si$ $M^{+\bullet}$ 302.1913, found 302.1912.

(2-Trimethylsilyl)-ethyl-9-oxo-nonanate (14). A chilled and well-stirred solution of $Al(OEtOMe)_2H_2$ (9.5 mL, 3.5 M in toluene) in dry THF (10 mL) was gradually treated with *N*-methylpiperazine (3.9 mL, 36.6 mmol). This reagent was then added dropwise with stirring to a cold solution (−50 to 70 °C) of the silyl ester **21** (1.5 g, 5 mmol) in THF (20 mL).[19] The flask was allowed to warm to −20 °C, while the progress of the reaction was monitored by GC-MS. The reaction was stopped by acidification with 2 N HCl and the product was extracted with dichloromethane (3 × 25 mL). After drying (Na_2SO_4) and removal of the solvent, the product was purified by chromatography on silica gel, using petroleum ether:ethyl acetate (9:1, v:v) for elution. Colorless liquid (710 mg, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (t, J = 1.89 Hz, 1H), 4.10−4.19 (m, 2H), 2.40 (dt, J = 1.89 Hz, J = 7.55 Hz, 2H), 2.25 (t, J = 7.55 Hz, 2H), 1.55−1.66 (m, 4H), 1.25−1.37 (m, 6H), 0.93−1.00 (m, 2H), 0.02 (s, 9H); ^{13}C NMR (100 MHz, CDCl3) δ 203.35, 174.55, 63.04, 44.50, 35.09, 29.64, 29.60, 29.56, 25.52, 22.64, 17.98, −0.84; IR (KBr, film) 2936, 2859, 2823, 2718, 1727, 1459, 1414, 1382, 1350, 1250, 1173, 1100, 1059, 1041, 936, 864, 836 cm^{-1}; EI-MS 272 ($M^{+\bullet}$, 0.1), 257 (0.6), 243 (1), 229 (10), 211 (19), 199 (11), 173 (24), 129 (23), 117 (21), 75 (56), 73 (100); EI-HRMS calcd for $C_{14}H_{28}SiO_3$ $M^{+\bullet}$ 272.1808, found 272.1815.

5-(1,3-Dioxolane-2-yl)-pentan-2-ol (18).[15] A well-stirred suspension of magnesium turnings (1.1 g, 45 mmol) in dry THF (10 mL) was treated at 30−35 °C with 1,2-dibromoethane (90 μL) and 2-(bromoethyl)-1,3-dioxolane (**17**) (2.4 mL, 20 mmol). After completion of the reaction, the mixture was cooled to −78 °C, and a solution of $CuBr(Me_2S)$ (0.75 g, 3.7 mmol) was added dropwise to the well-stirred solution. After 1 h propylene oxide (1 mL, 15 mmol) was added with a chilled syringe. Stirring was continued for 3.5 h while the reaction was allowed to come to −30 °C followed by another 20 h warm-up to 0 °C. After hydrolysis with satd aq NH_4Cl, extraction with dichloromethane, drying (Na_2SO_4), and evaporation of the solvent, the crude product was purified by chromatography on silica gel. Elution with petroleum ether:ethyl acetate (7:3, v:v) afforded **18** as a colorless liquid (1.39 g, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.85 (t, J = 4.72 Hz, 1H), 3.91−3.99 (m, 2H), 3.76−3.89 (m, 3H), 1.62−1.74 (m, 2H), 1.41−1.59 (m, 4H), 1.18 (d, J = 6.23 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 105.20, 68.53, 65.52, 39.76, 34.38, 24.10, 20.83; IR (KBr, film) 3415 (br), 2956, 2929, 2882, 1456, 1410, 1369, 1128, 1054, 1031, 943, 845 cm^{-1}; EI-MS 159 ($[M - H]^{+\bullet}$, 1), 143 (10), 129 (8), 112 (6), 99 (6), 81 (9), 73 (100), 57 (17); EI-HRMS calcd for $C_8H_{15}O_3$ $[M - H]^{+\bullet}$ 159.1021, found 159.1019.

Linolenic Acid 4-(1,3-Dioxolane-2-yl)-1-methyl-butyl Ester (19). A solution of linolenic acid (1 g, 3.8 mmol) in dry dichloromethane (10 mL) was treated with *N*,*N*-(dimethylamino)-pyridine (100 mg, 0.82 mmol) and 5-(1,3-dioxolane-2-yl)-pentane-2-ol (**18**, 450 mg, 2.8 mmol). To the chilled and stirred solution was added dicyclohexylcarbodiimide (598 mg, 2.9 mmol). After 5 min, stirring was continued for 3 h at rt. The urea was filtered off and the organic layer was washed with 0.5 N HCl and satd aq $NaHCO_3$. After drying (Na_2SO_4) the solvent was removed under reduced pressure. The ester was purified by chromatography on silica gel, using petroleum ether:ethyl acetate (9:1, v:v) for elution. Colorless liquid (637 mg, 54%). ^{1}H NMR (400 MHz, $CDCl_3$) 5.26−5.43 (m, 6H), 4.90 (sext, J = 6.23 Hz, 1H), 4.83 (t, J = 4.77 Hz, 1H), 3.90−3.99 (m, 2H), 3.80−3.88 (m, 2H), 2.76−2.84 (pt, 4H), 2.26 (t, J = 7.48 Hz, 2H), 1.99−2.11 (m, 4H), 1.39−1.70 (m, 8H), 1.24−1.38 (m, 8H), 1.20 (d, J = 6.42 Hz, 3H), 0.97 (t, J = 7.49 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.16, 132.64, 130.96, 128.97, 128.94, 128.39, 127.08, 105.06, 71.19, 65.54, 36.48, 35.38, 34.34, 30.27, 29.87, 29.80, 27.89, 26.30, 26.21, 25.74, 21.23, 20.62, 14.96; IR (KBr, film) 3010, 2932, 2855, 1730, 1652, 1460, 1373, 1245, 1181, 1135, 1098, 1057, 1030, 938, 719 cm^{-1}; EI-MS 420 ($M^{+\bullet}$, 43), 305 (25), 277 (29), 261 (20), 143 (100), 108 (57), 101 (48); EI-HRMS calcd for $C_{26}H_{44}O_4$ $M^{+\bullet}$ 420.3240, found 420.3239.

Linolenic Acid 1-Methyl-5-oxo-pentyl Ester (12). The dioxolane was cleaved by stirring **19** (750 mg, 1.79 mmol) in THF (50 mL) and 6 N HCl (25 mL) at 0 °C for 3 h. The mixture was allowed to warm to rt and stirred overnight. After extraction with dichloromethane and drying (Na_2SO_4), the solvent was removed under reduced pressure. Chromatography on silica gel, using petroleum ether:ethyl acetate (9:1, v:v) for elution, afforded **12** a colorless liquid (562 mg, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (t, J = 1.51 Hz,1H), 5.44−5.25 (m, 6H), 4.88 (qt, J = 6.3 Hz, J = 5.28 Hz, 1H), 2.83−2.74 (m, 4H), 2.49−2.41 (m, 2H), 2.27 (t, J = 7.50, 2H), 2.00−2.13 (m, 4H), 1.73−1.47 (m, 6H), 1.39−1.23 (m, 8H), 1.21 (d, J = 6.36 Hz, 3H), 0.97 (t, J = 7.50 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.41, 174.05, 132.61, 130.91, 128.97, 128.94, 128.43, 127.81, 70.69, 44.15, 35.95, 35.32, 30.23, 29.81, 29.77, 27.87, 26.30, 26.21, 25.70, 21.21, 20.58, 18.63, 14.89; IR (KBr, film) 3010, 2934, 2853, 2717, 1733, 1457, 1370, 1246, 1181, 1132, 715 cm^{-1}; EI-MS 376 ($M^{+\bullet}$, 10), 277 (11), 261 (20), 135 (5), 129 (25), 121 (7), 108 (15), 99 (100), 81 (64), 69 (15), 67 (14), 55 (35); EI-HRMS calcd for $C_{24}H_{40}O_3$ $M^{+\bullet}$ 376.2978, found 376.2978.

17-Linolenoyloxy-linoleic Acid (2-trimethylsilyl)-ethyl Ester (15). A suspension of propane-1,3-bistriphenylphosphonium bromide (**13**) (661 mg, 0.91 mmol) in dry THF (15 mL) was stirred at −78 °C, and a solution of $KN(SiMe_3)_2$ (3.8 mL, 0.5 M in hexane) was slowly added. To achieve complete deprotonation, the solution was allowed to warm to 0 °C for 10 min and was then recooled to −78 °C. The first carbonyl component, (1*S*)-linolenic acid 1-methyl-5-oxo-pentyl ester (**12**) (338 mg, 0.9 mmol) in 3 mL of dry THF, was added dropwise via a precooled (−78 °C) syringe. After 1 h the mixture was allowed to warm and was stirred for another 1 h at rt. After the mixture was recooled to −78 °C, the second carbonyl component, **14** (245 mg, 1 mmol) in dry THF (2 mL), was also slowly added via a precooled syringe. The reaction was allowed to warm and was stirred at rt for an additional 2 h. After hydrolysis with NH_4Cl, extraction with dichloromethane, and drying (Na_2SO_4), solvents were removed under reduced pressure. The pure ester **15** was obtained as a colorless liquid by chromatography on silica gel, using petroleum ether:ether (95:5, v:v) for elution (172 mg, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.24−5.45 (m, 10H), 4.85−4.95 (m, 1H), 4.11−4.19 (m, 2H), 2.69−2.86 (m, 6H), 2.27 (t, J = 7.55 Hz, 2H), 2.26 (t, J = 7.55 Hz, 2H), 1.95−2.13 (m, 8H), 1.23−1.67 (m, 24H), 1.20 (d, J = 6.04 Hz, 3H), 0.97 (t, J = 7.43 Hz, 3H), 0.66−1.00 (m, 2H), 0.04 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.71, 174.19, 132.65, 130.96, 130.91, 130.79, 130.29, 130.17, 129.23, 129.05, 128.97, 128.94, 128.51, 128.41, 127.81, 71.24, 63.05, 36.24, 35.41, 35.22, 30.28, 29.88, 29.82, 27.90, 27.64, 26.30, 26.22, 26.12, 25.76, 25.67, 21.24, 20.74, 18.01, 14.97, 1.70, −0.80; IR (KBr, film) 3010, 2928, 2855, 1734, 1455, 1377, 1245, 1172, 1135, 856, 833 cm^{-1}; EI-MS 656 ($M^{+\bullet}$, 2), 629 (26), 614 (6), 351 (83), 350 (100), 349 (42), 335 (55), 321 (7), 308 (11), 294 (7), 278 (67), 277 (51), 261 (46), 243 (9), 236 (14), 196 (25), 163 (16), 135 (48), 121 (44); EI-HRMS calcd for $C_{41}H_{72}O_4Si$ $M^{+\bullet}$ 656.5200 found 656.5176.

17-Linolenoyloxy-linoleic Acid (16). A solution of the (2-trimethylsilyl)-ethyl ester **15** (100 mg, 0.15 mmol) in THF (2.5 mL) was stirred at rt with a solution of tetrabutylammonium fluoride (2 mL, 1 M solution in THF) overnight. After acidification with HCl (10 mL, 2 N), the free acid **16** was extracted with dichloromethane. After drying (Na_2SO_4) and evaporation of the solvent under reduced pressure, the acid was purified by RP18 chromatography, using a binary gradient from H_2O:MeOH (30:70, v:v), to 100% MeOH (52 mg, 62%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.26−5.46 (m, 10H), 4.91 (sext, J = 6.38 Hz, 1H), 2.85−2.73 (m, 6H), 2.35 (t, J = 7.55, 2H), 2.26 (t, J = 7.43 Hz, 2H), 2.00−2.13 (m, 8H), 1.23−1.68 (m, 24H), 1.20 (d, J = 6.30 Hz, 3H), 0.97 (t, J = 7.55 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.58, 174.27, 132.68, 130.99, 130.89, 130.19, 129.29, 129.03, 128.99, 128.62, 128.47, 127.87, 71.32, 36.28, 35.45, 34.24, 30.30, 30.24, 29.88, 29.84, 29.84, 29.75, 29.72, 27.93, 27.90, 27.68, 26.36, 26.26, 26.14, 25.79, 25.42, 21.26, 20.72, 14.94; IR (KBr, film) 3010, 2928, 2855, 1734, 1711, 1460, 1373, 1245, 1181, 1130, 1085, 719 cm^{-1}; EI-MS 556 ($M^{+\bullet}$, 28), 294 (10), 278 (100), 277 (55), 236 (18), 222 (12), 135 (20), 113 (39); EI-HRMS calcd for $C_{36}H_{60}O_4$ $M^{+\bullet}$ 556.4492, found 556.4481.

***N*-(17-Linolenoyloxy-linoleoyl)-L-glutamine (7).** Free acid **16** (50 mg, 0.10 mmol) and triethylamine (11 mg, 0.11 mmol) were stirred at −10 °C under argon in dry THF (4 mL), and ethyl chloroformate (12 mg, 0.11 mmol) was added. After 5 min a solution of L-glutamine (30 mg, 0.20 mmol) in NaOH (2.8 mL, 0.3 N) was added, and the mixture was stirred at rt for 30 min. The solution was acidified with 2 N HCl and extracted with dichloromethane. The combined organic layers were dried (Na_2SO_4) and the solvent removed under reduced pressure. The conjugate product was purified by HPLC on RP-18, using a binary gradient of acetonitrile:water (70:30, v:v), to pure acetonitrile (49.6 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.09 (s, br, 1H), 7.07 (s, br, 1H), 6.33 (s, br, 1H), 5.98 (s, br, 1H), 5.27−5.45 (m, 10H), 4.91 (sext, J = 6.32 Hz, 1H), 4.47 (q, J = 6.09 Hz, 1H), 2.74−2.87 (m, 6H), 2.55−2.64 (m, 1H), 2.39−2.48 (m, 1H), 2.26 (t, J = 7.41 Hz, 2H), 2.25 (t, J = 7.41 Hz, 2H), 2.15−2.24 (m, 2H), 1.99−2.13 (m, 8H), 1.24−1.68 (m, 24H), 1.20 (d, J = 6.17 Hz, 3H), 0.98 (t, J = 7.53 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.23, 175.42, 174.33, 173.85, 132.68, 130.99, 130.90, 130.20, 129.29, 129.04, 129.00, 128.62, 128.48, 127.87, 71.37, 53.22, 37.09, 36.26, 35.47, 32.63, 30.30, 29.90, 29.88, 29.84, 29.82, 28.34, 27.93, 27.68, 26.37, 26.36, 26.27, 26.20, 26.12, 25.80, 21.26, 20.72, 14.93; IR (KBr, film) 3429, 3327, 3210, 3013, 2959, 2927, 2858, 1731, 1657, 1542, 1456, 1423, 1260, 1188, 1098, 1026, 801 cm^{-1}; APCI-MS 685 (100), 407 (16), 261 (2), 147 (1), 130 (1); ESI-HRMS calcd for $C_{41}H_{69}N_2O_6$ $[M + H]^+$ 685.5155, found 685.5143.

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Supporting Information Available: Mass spectra and ^{1}H and ^{13}C NMR spectra of compounds **7**, **12**, **14**, **15**, **16**, **18**, **19**, and **21**. This material is available free of charge via the Internet at http://pubs.acs.org.

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