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TETRAHEDRON

Biosynthesis of Epilachnene, a Macrocyclic Defensive Alkaloid of the Mexican Bean Beetle[1]

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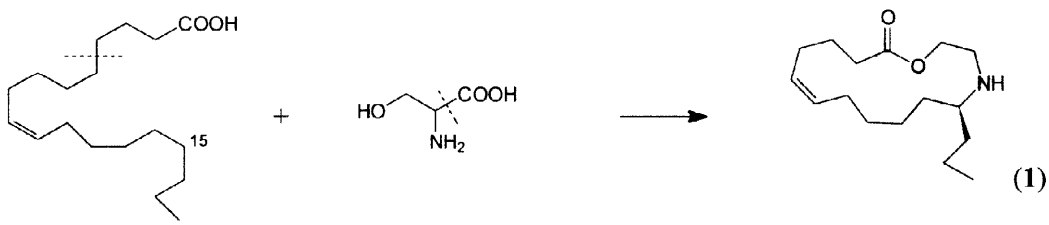
Abstract: The carbon skeleton of the azamacrolide epilachnene, the principal defensive alkaloid of *Epilachna varivestis* pupae, can be derived from oleic acid and serine. Analytical evidence from three experiments in which insects were fed [$^{2}H_{35}$]octadecanoic acid, (*Z*)-9-[11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-$^{2}H_{17}$]octadecenoic acid and (*Z*)-9-[11,11,12,12,13,13,14,14,16,16,17,17,18,18,18-$^{2}H_{15}$]octadecenoic acid, indicates that only the C-15 methylene group of oleic acid is involved in the mechanism leading to carbon-nitrogen bond formation in epilachnene. Support for this scheme is provided by the observation of an unusual ion at *m/z* 170 in the electron-ionization mass spectrum of epilachnene, rationalized as CH_2=CH-COO-CH_2-CH_2-NH^+=CH-CH_2-CH_2-CH_3.

Keywords: Biosynthesis; Natural products; Mass spectrometry; Insects; Labelling.

INTRODUCTION

Elaborate defensive mechanisms involving exocrine chemicals contribute significantly to the fitness of insects.[4] Although a large number of defensive compounds have been identified[5] and their behavioral roles examined, only a handful of investigations has focused on the determination of biosynthetic pathways leading to these allomones. In particular, the biosynthetic pathways leading to the production of the large and structurally varied defensive alkaloids of beetles remain virtually unexplored.[6] The azamacrolides, a small group of macrocyclic alkaloids produced by the pupa of the Mexican bean beetle, *Epilachna varivestis* (Coccinellidae),[7] pose an especially interesting biosynthetic question. Exploratory biosynthetic experiments with ^{2}H, ^{13}C, and ^{15}N-labeled precursors showed that the beetles can synthesize epilachnene (**1**), the most abundant of these alkaloids, from oleic acid and serine as outlined in Scheme 1.[8]



(1)

Scheme 1: Biosynthesis of epilachnene from oleic acid and serine.

In the course of this process, the oleic acid loses four carbon atoms (C-1—C-4), presumably as a consequence of two β-oxidations, and the serine moiety is decarboxylated. Of particular interest is the

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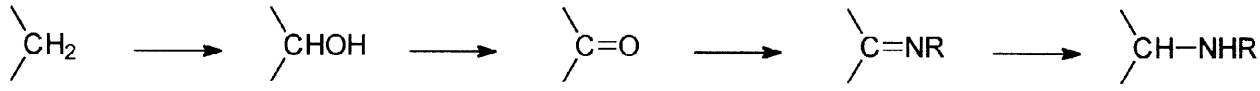
mechanism whereby the C-15 methylene group in oleic acid is functionalized so as to introduce a carbon-nitrogen bond in place of an unactivated carbon-hydrogen bond. This step deserves special attention since the majority of alkaloids produced by coccinellid beetles appear to result from analogous aminations of fatty acid chains.[9]

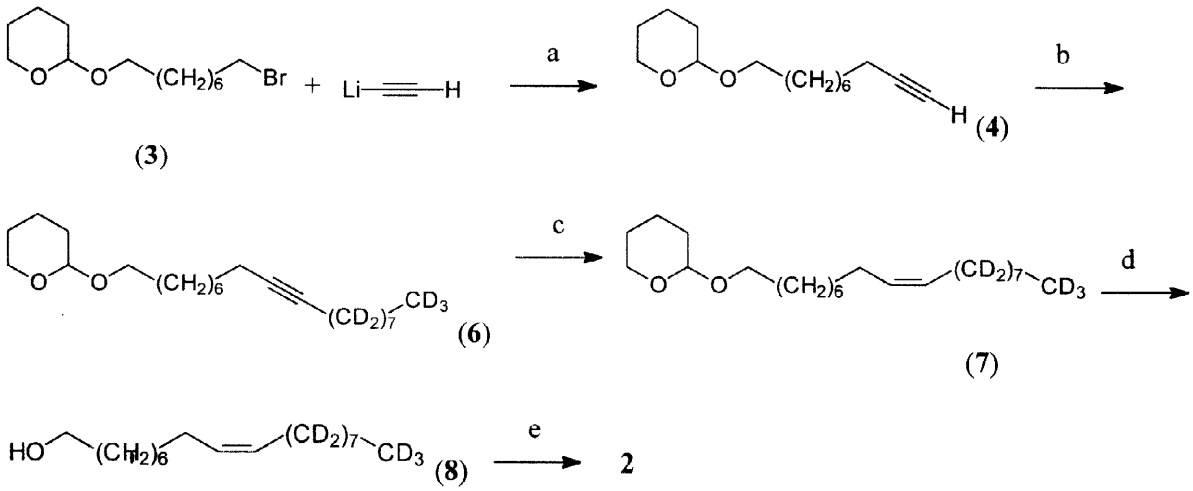
A variety of pathways can be envisioned for this transformation. Hydroxylation of oleic acid at C-15 (or at the corresponding site after chain shortening), oxidation to the corresponding ketone, Schiff base formation with serine or a serine-related amine, and imine reduction, as outlined in Scheme 2, would be the most straightforward possibility. This sequence requires loss of both hydrogen atoms at C-15 of the oleic acid precursor. Alternatively, nucleophilic displacement of a derivatized C-15 hydroxyl group, or direct insertion of a serine-derived nitrene into one of the carbon-hydrogen bonds at C-15, would necessitate the loss of only a single C-15 hydrogen atom, while a pathway involving an initial dehydrogenation to generate a C-14 or C-15 double bond would require loss of one hydrogen atom from either C-14 or C-16 along with one from C-15. We have now completed deuterium labeling and feeding experiments which support expectations based on Scheme 2, and which exclude the other above-mentioned alternatives.



Scheme 2: Postulated methylene amination sequence.

RESULTS AND DISCUSSION

A sample of (*Z*)-9-[11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-$^2H_{17}$]octadecenoic acid (**2**) was synthesized as shown in Scheme 3. Protected alkynol **4**, prepared by alkylation of lithium acetylide with halide **3**, was coupled[10] with perdeuterio-*n*-octylbromide **5**, and the carbon-carbon triple bond of the product (**6**) was hydrogenated using P2-Ni-catalyst.[11] THP-protected alkenol **7** obtained in this way was deprotected to form (9*Z*)-[$^2H_{17}$]octadecen-1-ol (**8**), which was oxidized with pyridinium dichromate[12] to yield the desired **2** (Scheme 3).



Scheme 3: (a) DMSO, 4-8 °C; (b) *n*-BuLi, *n*-[$^2H_{17}$]octyl bromide (**5**) in DMPU, 20 °C; (c) P2 Ni/H_2; (d) Dowex 50WX, MeOH; e) PDC/DMF.

An experiment in which (*Z*)-9-[11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-$^2H_{17}$]octadecenoic acid (**2**) was fed to *E. varivestis* larvae provided definitive evidence for the loss of two deuterium atoms in the course of epilachnene biosynthesis. This extensively deuteriated oleic acid proved to be an excellent precursor of epilachnene; the gas chromatogram (Fig. 1B) of the alkaloids produced in this experiment showed a well-resolved peak corresponding to the deuteriated product with an intensity of about 20% of that of the unlabeled **1**.

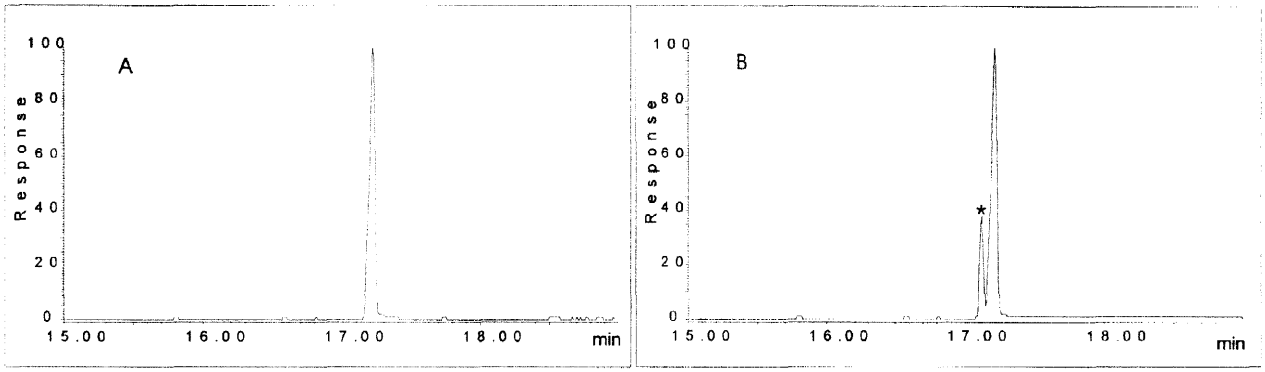
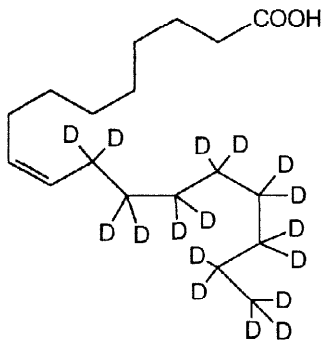
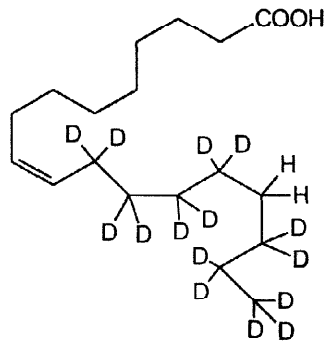


Fig. 1. Reconstructed gas chromatograms obtained by GC-MS analysis of *E. varivestis* pupal secretion. (A) Larvae fed on diet enriched with oleic acid; (B) larvae fed with diet enriched with [$^2H_{17}$]oleic acid (**2**). The asterisk denotes deuterium-labeled epilachnene.

While the molecular ion of epilachnene appears at *m/z* 267 (**Fig. 2A**), the mass spectrum of this deuteriated epilachnene showed a molecular ion peak at *m/z* 282 (**Fig. 2B**), indicating that fifteen of the seventeen deuterium atoms present in precursor **2** were retained. This observation excludes pathways predicting loss of a single deuterium atom, but leaves open the question of whether both deuteriums were lost from C-15, or whether only one was lost from C-15, and the second from an adjacent position.



2



9

To make this distinction, a sample of (*Z*)-9-[11,11,12,12,13,13,14,14,16,16,17,17,18,18,18-$^2H_{15}$]octadecenoic acid (**9**) was synthesized stating from THP-protected alkynol **4** as shown in Scheme 4. The lithium salt of **4** was coupled with 4-trimethylsilyloxy-1-iodobutane (**10**),[13] and the TMS group was removed[14] from the resulting **11** to give alcohol **12**, which was hydrogenated[11] to yield the THP-protected alkenol **13**. This product was converted into the corresponding iodide (**14**)[15] which was coupled with CD_3-$(CD_2)_2$-CH_2MgBr.[16] The product (**15**) was deprotected and oxidized[12] to yield the desired deuteriated acid **9**.

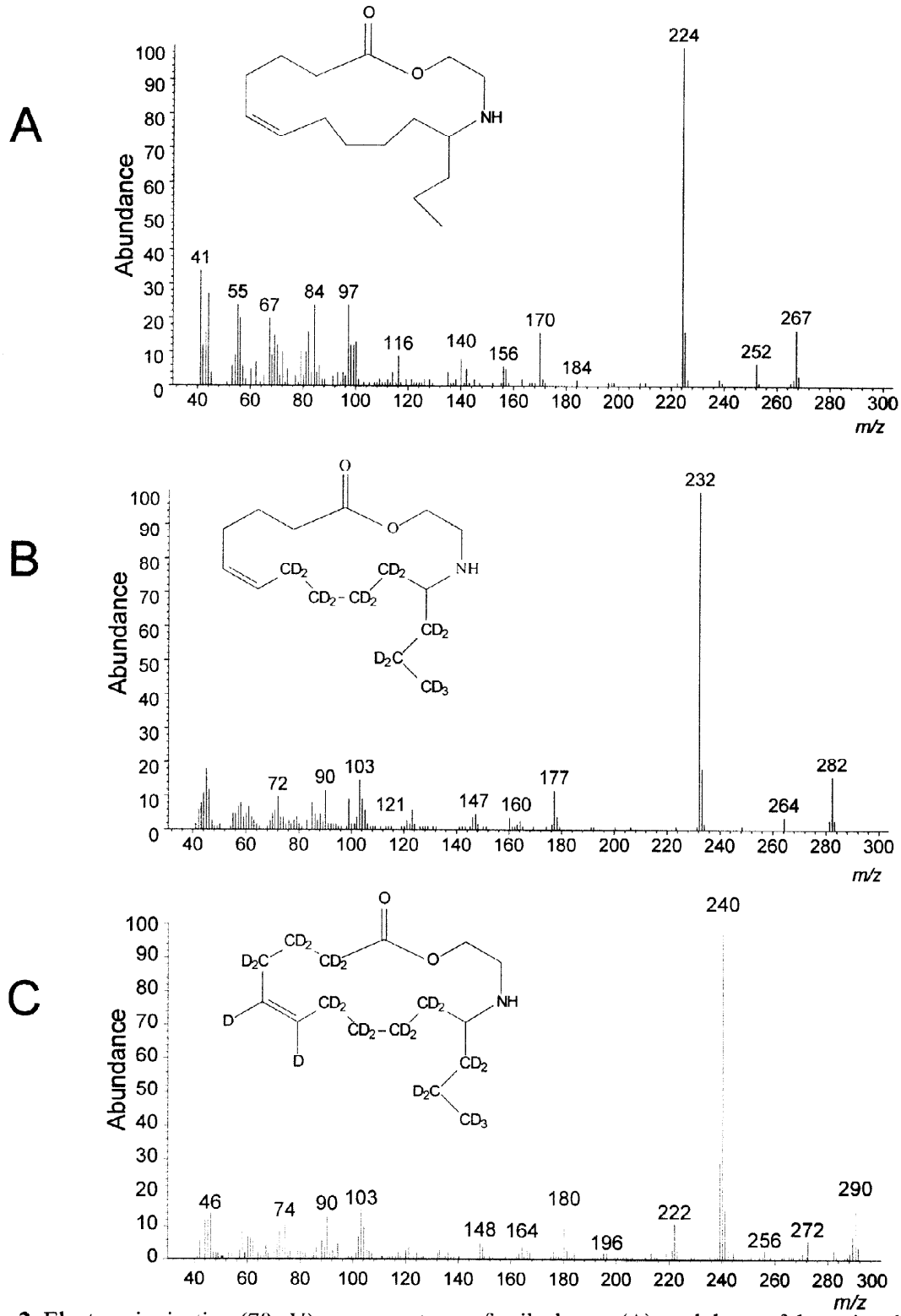
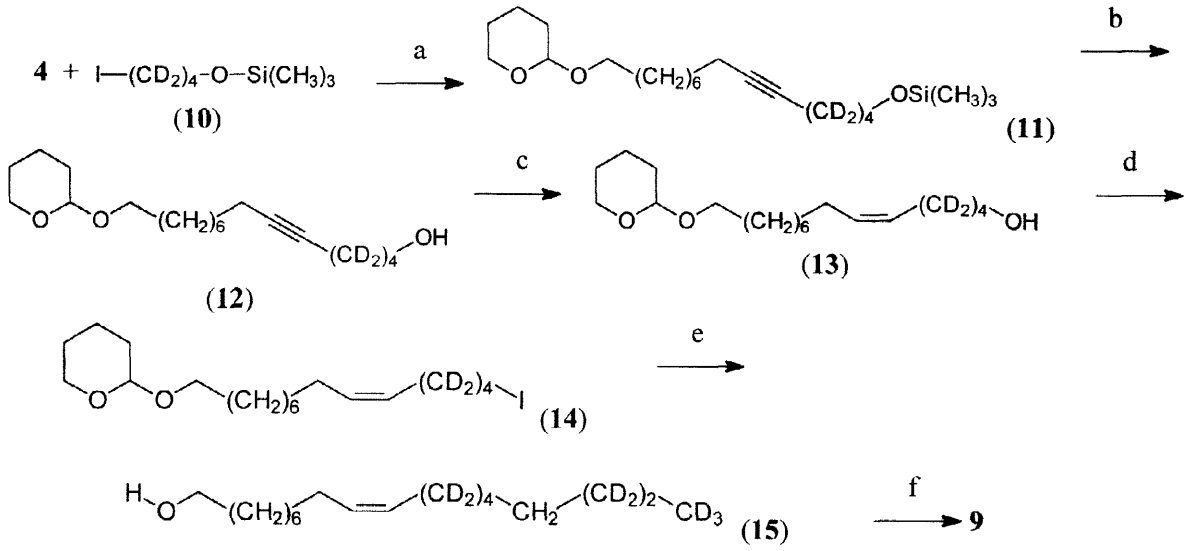


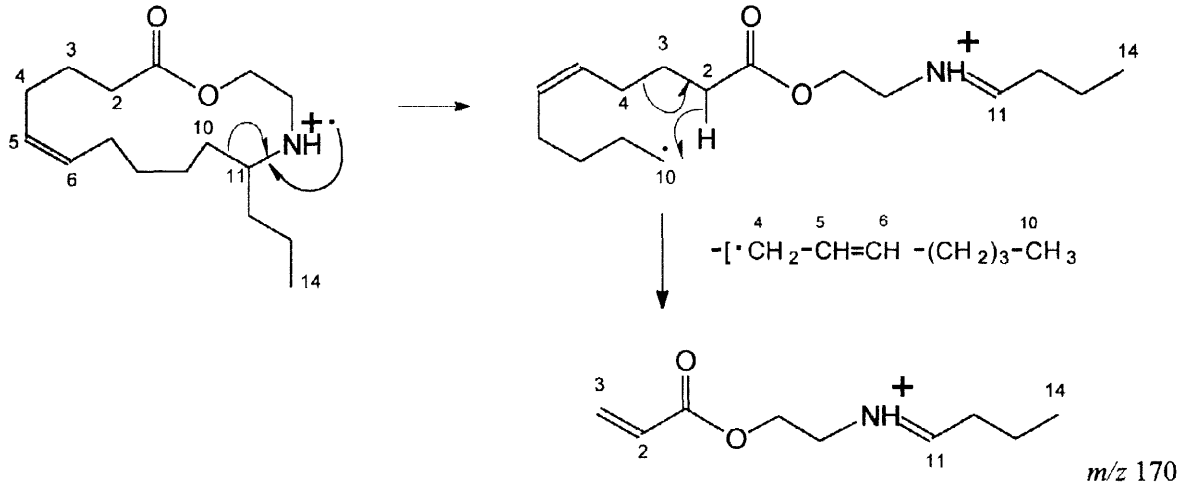
Fig. 2. Electron-ionization (70 eV) mass spectrum of epilachnene (A), and those of deuteriated epilachnenes derived from pupa reared on a (*Z*)-9-[11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-$^{2}H_{17}$]octadecenoic acid (**2**) (B), and [$^{2}H_{35}$]octadecanoic acid-enriched (C) diets.



Scheme 4: (a) *n*-BuLi, **10** in HMPA; (b) TBAF; (c) P2 Ni/H_2; (d) 1) TsCl/Py; 2) NaI/acetone; (e) 1) CD_3-$(CD_2)_2$-CH_2MgBr, $Li_2Cu(CN)Cl_2$, THF; 2) TsOH/MeOH; (f) PDC/DMF.

A feeding experiment utilizing this specifically deuteriated acid (**9**) gave a most useful result. The labeled epilachnene produced from this precursor showed no loss of deuterium (*m/z* 282), demonstrating that no changes occurred at either C-14 or C-16. These findings are in full accord with the pathway proposed in Scheme 2. As it turns out, these experiments also clarify an unusual fragmentation observed in the electron-ionization mass spectra of epilachnene and related azamacrolides, and thereby provide complementary evidence in support of our biosynthetic conclusions.

The mass spectrum of epilachnene shows a prominent, unexpected fragment ion at *m/z* 170. High-resolution MS shows the elemental composition of this ion to be $C_9H_{16}NO_2^+$ [obsd. mass 170.1197; calc for $C_9H_{16}NO_2^+$ 170.1181], resulting from the loss of a C_7H_{13} radical. We considered this fragmentation to proceed by an initial α-cleavage (of the C-10/C-11 bond), which opens the large ring, followed by a remote hydrogen atom transfer from C-2 and fission of the C-3/C-4 bond, which leads to loss of a C_7H_{13} allylic radical (Scheme 5). This mechanism results in loss of C-4 through C-10, along with all of the hydrogen atoms originally bound to these carbon atoms, plus the one hydrogen atom transferred from C-2.



Scheme 5: Proposed fragmentation pathway for the formation of the *m/z* 170 ion.

Table 1. Electron-ionization mass spectra (70 eV) of epilachnene and some related azamacrolides.

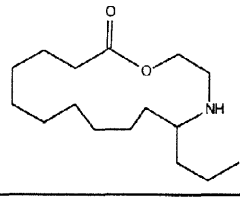
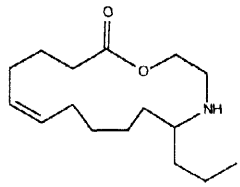
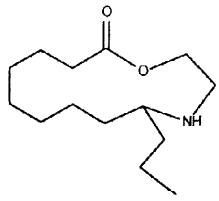
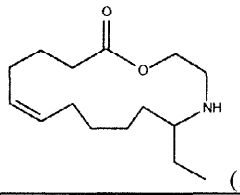
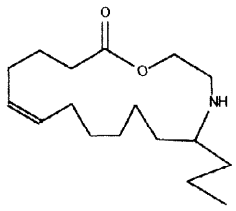
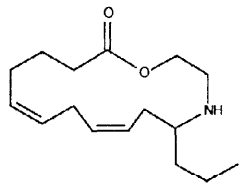
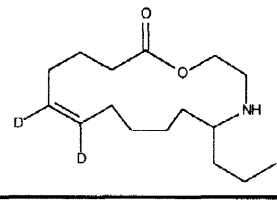
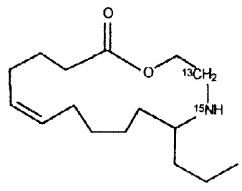
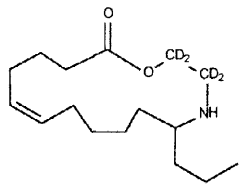
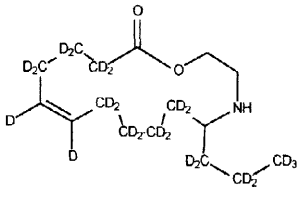
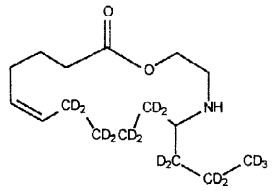
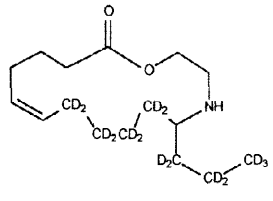
compound	structure	key fragment ions [*m/z*(%)]
epilachnane (dihydroepilachnene)	 (**16**)	269 (M^+,5), 254(5), 226(100), **170**(13), 116(12), 99(14), 97(16), 84(16), 82(29), 72(11), 71(4), 70(13), **69**(19), **68**(8), 76(10), 54(19), 55(31), 41(50).
(5Z)-11-propyl-12-azacyclotetradec-5-en-14-olide (epilachnene)	 (**1**)	267(M^+,10), 252,(6), 225 (16), 224(100), **170**(19), 157(5), 156(8), 140(8), 116(13), 97(31), 84(29), 67(28), 56(33), 55(35), 44(28), 43(24), 42(20), 41(56).
9-propyl-10-azacyclododecan-12-olide (synthetic[17] and natural)	 (**17**)	241(M^+,8), 226(7), 210(8), 199(16), 198(100), **170**(17), 142(8), 116(12), 99(20), 97(22), 82(37), 72(44), 55(32).
(5Z)-11-ethyl-12-azacyclotetradec-5-en-14-olide (norepilachnene)	 (**18**)	253(M^+,30), 225(15), 224(100), 198(12), **156**(25), 140(15), 100(30), 83(73), 70(78), 68(87), 56(50).
(5Z)-12-propyl-13-azacyclopentadec-5-en-15-olide (homoepilachnene)	 (**19**)	281(M^+,22), 238(100), 224(10), 184(8), **170**(8), 112(15), 98(50), 74(30), 58(30).
(5Z,8Z)-11-propyl-12-azacyclotetradeca-5,8-dien-14-olide (epilachnadiene)	 (**20**)	265(M^+,45), 250(17), 222(30), **170**(32), 116(70), 82(100).
Labeled epilachnene from (*Z*)-9-[9,10-$^2H_2$]octadecenoic acid-fed insects	 (**21**)	269(M^+,11), 254(6), 227(16), 226(100), **170**(12), 157(6), 156(5), 141(5), 116(10), 97(18), 84(21), 82(16), **69**(18), 56(22), 44(19), 41(22).

Table 1. continued

labeled epilachnene from L-[2-^{13}C,^{15}N]serine-fed insects	 (22)	269 (M^+,12), 254(5), 226(100), **172**(13), 144(3), 102(12), 73(2), 46(27).
(5Z)-11-propyl-12-[13,13,14,14-$^2H_4$]azacyclotetradec-5-en-14-olide (synthetic [$^2H_4$]epilachnene)	 (23)	271(M^+,12), 256(6), 228(100), **174**(19), 161(8), 160(10), 146(9), 144(8), 120(14), 104(17), 103(17), 100(23), 88(10), 86(36), 85(17), 81(11), 79(14), 72(23), 67(24), 55(32), 48(31), 41(40).
labeled epilachnene from perdeuterated stearic acid-fed insects	 (24)	290(M^+,15), 272(5), 256(4), 240(100), 222(11), **180**(10), 166(3), 164(3), 149(5), 148(5), 103(14), 90(13), 74(10), 58(9), 46(16).
(Z)-9-[11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-$^2H_{17}$]octadecenoic acid-fed insects	 (25)	282(M^+,16), 264(5), 232(100), **177**(11), 103(13), 90(10), 72(8), 46(10), 45(15)
(Z)-9-[11,11,12,12,13,13,14,14,16,16,17,17,18,18,18-$^2H_{15}$]octadecenoic acid-fed insects	 (26)	282(M^+,11), 264(4), 232(100), **177**(27), 103(41), 90(35), 72(15), 46(39), 45(45)

If this fragmentation mechanism is valid, then an *m/z* 170 peak would be expected to appear in the spectra of all azamacrolides bearing a propyl side chain. From the data listed in Table 1, an *m/z* 170 peak is, in fact, observed in the spectra of epilachnane (**16**), 9-propyl-10-azacyclododecan-12-olide (**17**), homoepilachnene (**19**), and epilachnadiene (**20**). Moreover, the spectrum of norepilachnene (**18**), which has an ethyl in place of a propyl side chain, shows a corresponding peak at *m/z* 156. It is interesting to note that the *m/z* 170 peak is relatively more prominent in the spectrum of epilachnadiene (**20**), presumably because both the radicals formed in the initial ring cleavage and in the last step are allylic. A synthetic sample of (5Z)-11-propyl-12-[13,13,14,14-$^2H_4$]azacyclotetradec-5-en-14-olide ([13,13,14,14-$^2H_4$]epilachnene) (**22**) showed the corresponding ion at *m/z* 174 (Table 1) (accurate mass 174.1431; calc. for $C_9H_{12}D_4N_1O_2$ 174.1432), in accord with the proposed mechanism.

Based on this mechanism, the corresponding ions observed in the mass spectra of the labeled epilachnenes derived from the isotopically labeled precursors used in our biosynthetic experiments can be rationalized. For example, while the mass spectrum of labeled epilachnene (**21**) produced by (Z)-9-[9,10-$^2H_2$]octadecenoic acid-fed insects showed a molecular ion and a base peak at two mass units

higher than that of epilachnene, the *m/z* 170 peak remained unchanged (Table 1). In contrast, the spectrum of labeled epilachnene (**22**) derived from L-[2-^{13}C,^{15}N]serine-fed insects showed the corresponding peak at *m/z* 172 (Table 1).

Recently, we showed by a direct gas chromatographic comparison of the (*S*)-α-methoxy-α-trifluoromethylphenylacetyl amide (MTPA amide) of natural epilachnene with the corresponding amides of independently synthesized (*R*)- and (*S*)-epilachnene, that natural epilachnene occurs as a single enantiomer with the *S* configuration at its one chiral center [(***S***)-**1**].[18] This absolute configuration must be established by the stereospecific reduction of the imine intermediate produced by the condensation of the oleic acid and serine-derived moieties.

Finally, evidence supporting both the proposed biosynthetic pathway and the mass spectral fragmentation mechanism was obtained by an experiment in which [$^{2}H_{35}$]stearic acid was fed to larvae of *E. varivestis*. Although the incorporation of stearic acid into epilachnene was not as efficient as that of oleic acid, the insects were able to utilize this completely saturated acid as an epilachnene precursor. The labeled epilachnene (**24**) derived from this experiment showed a molecular ion at *m/z* 290 and the base peak at *m/z* 240 resulting from the loss of the perdeuteriopropyl side chain (**Fig. 2C**). It follows that twelve deuterium atoms are lost in the process: eight deuterium atoms are removed during the fatty acid chain shortening, two in the formation of the double bond, and two when the alkyl chain is aminated. Moreover, the mass spectrum of **24** showed a peak at *m/z* 180 (Table 1), which corresponds to the ten-deuterium-atom-bearing fragment ion predicted by Scheme 5. Based on all these results, the broad outlines of azamacrolide biosynthesis in the Mexican bean beetle from fatty acid precursors and serine are now clear.

EXPERIMENTAL

Instrumentation. NMR spectra were recorded on Unity-200 (^{1}H, 200 MHz), Unity-400 (^{1}H NMR, 400 MHz; ^{13}C NMR, 100.6 MHz), and Unity-500 (^{1}H NMR, 500 MHz; ^{13}C NMR, 125.7 MHz) Varian instruments as $CDCl_3$ or C_6D_6 solutions at room temperature. Chemical shifts, given in ppm, are expressed as δ values measured from the residual $CHCl_3$ signal (7.26 ppm). Vapor phase infrared spectra were recorded using a Hewlett-Packard (HP) 5965A IRD coupled to a HP 5890 GC. For GC analysis, a fused-silica capillary column (30 m x 0.25 mm) coated with DB-5 (0.25 μm) fitted in a HP 5890 GC equipped with a flame-ionization (FID) detector was used. Low-resolution electron-ionization mass spectra were obtained using a Hewlett-Packard (HP) 5890 gas chromatograph linked to a HP 5970 mass selective detector (MSD). Dichloromethane extracts were introduced by splitless injection. Analyses were performed using a 25 m x 0.22 mm fused-silica column coated with DB-5 (J&W Scientific, Folsom, CA). The oven temperature was kept at 60 °C for 4 min and programmed to 270 °C at 15 °C/min. High-resolution GC-MS data were obtained using a VG 70-VSE instrument.

Chemicals. [$^{2}H_7$]Butyric acid (98% ^{2}H), [$^{2}H_{17}$]octan-1-ol (98% ^{2}H), and [$^{2}H_{35}$]octadecanoic acid were purchased from Cambridge Isotopes (Andover, MA). [$^{2}H_8$]Tetrahydrofuran (99.5% ^{2}H) was from Aldrich Chemicals (Milwaukee, IL). 9-Propyl-10-azacyclododecan-12-olide was available from an earlier synthesis.[17] 1-Bromo-8-(tetrahydropyran-2-yloxy)-octane (**3**) was prepared from the corresponding bromoalcohol by stirring with 3,4-dihydro-(2*H*)-pyran and a catalytic amount of pyridinium tosylate (93% yield).

*Synthesis of (Z)-9-[11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-$^{2}H_{17}$]octadecenoic acid: 10-(Tetrahydropyran-2-yloxy)-1-decyne (**4**).* A suspension of lithium acetylide-ethylendiamine complex (90%, 2.0 g, 20 mmol) in dry DMSO (10 ml) was treated with a dry DMSO solution (10 ml) of bromide **3** (2.75 g, 9.4 mmol) at 4-8 °C over a period of 1.5 h.[19] After 12 h of stirring at room temperature,

the reaction mixture was quenched with cold water (5 ml). After the exothermic reaction ceased, an additional portion of water (10 ml) with hexane (5 ml) was added. The organic phase was separated and the aqueous layer was extracted with hexane (3 × 20 ml). The combined solvent extract was washed with brine (20 ml), dried over $MgSO_4$ and distilled to yield 2.00 g (90 % yield) of **4** as a colorless oil (b.p. 170 - 180 °C/0.25 torr). ^{1}H NMR ($CDCl_3$) δ: 1.8-1.3 (18H, m), 1.94 (1H, t, J = 2.6 Hz, ≡C-H), 2.18 (2H, dt, J = 2.6, 6.8 Hz, CH_2-C≡), 3.40 (2H, m, CH_2[1]), 3.80 (2H, m, CH_2[6′]), 4.58 (1H, bs, CH[2′]); EIMS *m/z* (%): 237(1) [M^+-1], 101 (27), 95 (11), 85 (100), 81 (17), 79 (7), 67 (20), 57 (11), 56 (19), 55 (25), 43 (14), 41 (34).

1-Bromo-[$^2H_{17}$]octane (5). An ice-cold solution of 1-[$^2H_{17}$]octanol (0.44 g, 3 mmol) and Ph_3P (0.83 g, 3.15 mmol) in dry CH_2Cl_2 (4 ml) was treated with CBr_4 (1.00 g, 3.15 mmol) and stirred at room temperature for 3 h.[20] The reaction mixture was diluted with pentane (5 ml) and concentrated under reduced pressure. The white slurry formed was treated three times with pentane (3 × 5 ml) under vigorous mixing. Combined pentane extracts were passed thorough a short column of silica gel to remove traces of Ph_3P=O. The solvent was removed, and Kugelrohr distillation of the resulting oil afforded 0.54 g (86% yield) of bromide **5** (b.p. 155-160°C/15 torr). EIMS *m/z* (%): 210 (3) [M^+], 208 (2) [M^+], 161 (7), 160 (4), 159 (14), 145 (99), 144 (31), 143 (96), 142 (17), 82 (49), 81 (25), 66 (61), 75 (28), 64 (21), 62 (69), 61 (14), 50 (72), 49(30), 48 (19), 46 (100), 45 (19), 42 (35); IR cm^{-1}(percent transmittance): 2208 (98.79), 2105 (99.28), 1082 (99.78), 993 (99.50).

1-(Tetrahydropyran-2-yloxy)-[11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-$^2H_{17}$]octadec-9-yne (6). A solution of acetylene (**4**) (1.79 g, 0.78 mmol) in dry THF (0.5 ml) was metallated at -40 °C with a *n*-BuLi in hexanes (2 M, 380 μl, 0.76 mmol) over a period of 10 min. The mixture was warmed to 0 °C over a period of 0.5 h, stirred at this temperature for 0.5 h, and kept at 20 °C for 40 min. The mixture was cooled on an ice bath and a solution of 1-bromo-[$^2H_{17}$]octane (**5**, 105 mg, 0.5 mmol) in DMPU (0.3 ml) was added over a period of 45 min. The deep-brown reaction mixture that resulted was stirred at 0 °C for 4 h, and at 20 °C for 10 h. The reaction was quenched by adding a sat. NH_4Cl solution (10 ml), and the mixture was extracted with pentane/ether (9/1, v/v, 3 × 15 ml). After the usual work-up and silica gel (50 g) flash chromatography, 100 mg (54% yield) of **6** was isolated. ^{1}H NMR ($CDCl_3$) δ: 1.3-1.8 (18H, m), 2.14 (2H, t, J = 6.0 Hz, CH_2-C≡), 3.40 (2H, m, CH_2[1]), 3.80 (2H, m, CH_2[6′]), 4.58 (1H, bs, CH[2′]); EIMS *m/z* (%): 367 (1) [M^+-1], 237 (3), 101 (25), 85 (100), 71 (15), 69 (16), 55 (70), 41 (33).

(Z)-9-[11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-$^2H_{17}$]Octadecenoic acid (2). A solution of acetylene **6** (84.5 mg, 0.23 mmol) in deoxygenated EtOH (3 ml) was added to P2 Ni catalyst [prepared from 11 mg of $Ni(OAc)_2$] suspension in deoxygenated EtOH (7 ml) under hydrogen. The resulting black suspension was stirred under a slight excess pressure of hydrogen. After 1.5 h, the catalyst was filtered off and the solvent was evaporated. The residue oil was dissolved in CH_3OH (5 ml), and acidic ion-exchange resin Dowex 50W (30 mg) was added. After 12 h an additional portion of the resin (30 mg) was added. After 12 h of stirring, the resin was filtered, the solvent evaporated, and the residue was purified on a short silica gel column (10 g) providing (9Z)-[$^2H_{17}$]octadec-9-en-1-ol (**8**) (64.3 mg). The alcohol was dissolved in dry DMF (1.5 ml) and PDC (250 mg) was added.[12] The brown solution thus obtained was stirred for 48 h at room temperature. The resulting mixture was extracted with pentane/ether (9/1, v/v, 3 × 2 ml), and the combined extract was filtered thorough a Celite pad (3 g). The solution obtained was evaporated, and the residue was left for 12 h under vacuum. The residue oil was dissolved in aq. NaOH (1 M, 3 ml) and the solution was extracted with hexane (3 × 7 ml). The aqueous phase was treated with citric acid (1 g) and the free acid was extracted with ether (3 × 3 ml). The product was purified by silica gel (10 g) chromatography affording 44 mg (64 % yield from **6**) of **2** as a viscous oil. ^{1}H NMR ($CDCl_3$) δ: 1.38-1.30 (8H, m, CH_2[4, 5, 6, 7]), 1.63 (2H, tt, J = 4 x 7.3 Hz, CH_2[3]), 2.00 (2H, dt, J = 5.2, 6.0, 6.0, CH_2[8]), 2.35 (2H, t, J = 7.3, 7.5 Hz, CH_2[2]), 5.34 (2H, m, -CH=CH-); ^{13}C NMR ($CDCl_3$) δ: 24.8 (C-3), 27.4 (C-7), 29.3 (C-4*), 29.3 (C-6*), 29.4 (C-8*), 29.9 (C-5), 34.3 (C-2), 130.0 (C-9), 130.2 (C-10), 180.0 (C-1); EIMS *m/z* (%): 300 (M^++1, 2), 299 (M^+, 8), 298 (M^+-1, 1), 281 (M^+-18, 14), 280 (20), 237 (7), 185 (5), 123 (21), 110 (31), 98 (52), 84 (56), 83 (55), 73 (58), 60 (72), 55 (100), 41 (88): methylester: 313 (M^+, 20).

Synthesis of (Z)-9-[11,11,12,12,13,13,14,14,16,16,17,17,18,18,18-$^2H_{15}$]octadecenoic acid (9): *14-(Tetrahydropyran-2-yloxy)-[1,1,2,2,3,3,4,4,-$^2H_8$]tetradec-5-yl-1-ol (12).* Trimethylsilyl iodide (1.71 ml, 12 mmol) was added dropwise to [$^2H_8$]THF (1.5 ml, 15 mmol) cooled on an ice bath *via* a septum over a period of 0.3 h. The brown solution containing 4-trimethylsiloxy-1-iodobutane (**10**) was stirred 0.3 h at room temperature and used directly in the next step. An ice cold solution of acetylene **4** (2.8 g, 10 mmol) in dry THF (10 ml) was lithiated with *n*-BuLi in hexanes (1.6 M, 6.9 ml, 11 mmol) at 4 to 8 °C over a period of 0.5 h. The mixture was stirred for 0.5 h, and the iodide **10** diluted with HMPA (15 ml) was added over a period of 1 h at *ca* 5 °C. The colorless reaction mixture was poured into ice cold H_2O (15 ml) and extracted with hexane (3 × 25 ml). The combined extract was washed with H_2O (5 ml), and brine (5 ml), and dried. Flash chromatography on silica gel (100 g) with hexane/ethyl acetate mixtures afforded a 80:20 mixture (determined by ^1H NMR)(3.31 g, 85%) of the TMS derivative of **4** and **11**.
1-Trimethylsilyl-10-(tetrahydropyran-2-yloxy)-1-decyne (TMS derivative of **4**): ^1H NMR ($CDCl_3$) δ: 0.03 [9H, s, $Si(CH_3)_3$)], 1.3-1.8 (18H, m), 2.17 (2H, t, J = 7.1 Hz, CH_2-C≡), 3.40 (2H, m, CH_2[10]), 3.80 (2H, m, CH_2[6′]), 4.54 (1H, bs, CH[2′]); EIMS *m/z* (%): 310 (M^+, 0.1), 295 (1.5), 209 (1), 173 (12), 159 (3), 135 (4), 101 (12), 85 (100), 75 (24), 73 (50), 67 (10), 59 (10), 55 (9), 41 (15).
O-Trimethylsilyl-14-(tetrahydropyran-2-yloxy)-[1,1,2,2,3,3,4,4,-$^2H_8$]tetradec-5-yl-1-ol (**11**): ^1H NMR ($CDCl_3$) δ: 0.11 [9H, s, $Si(CH_3)_3$)], 1.3-1.8 (18H, m), 2.17 (2H, t, J = 7.1 Hz, CH_2-C≡), 3.40 (2H, m, CH_2[14]), 3.80 (2H, m, CH_2[6′]), 4.54 (1H, bs, CH[2′]); EIMS *m/z* (%): 390 (M^+, 0.1), 305 (M^+-85, 1), 191 (6), 177 (18), 173 (9), 159 (9), 133 (17), 101 (7), 85 (100), 76 (10), 75 (10), 73 (22), 67 (10), 43 (9), 41 (12).

(5Z)-14-(Tetrahydropyran-2-yloxy)-[1,1,2,2,3,3,4,4-$^2H_8$]tetradec-5-en-1-ol (13). The 80:20 mixture from the above experiment (3.25 g) was dissolved in THF (30 ml) and tetrabutylammonium fluoride (1M, 10 ml) was added. The mixture was stirred for 5 h and the solvent was evaporated. The resulting yellow oil was dissolved in ether (30 ml) and the solution washed with H_2O (2 × 30 ml) and brine (10 ml). The crude product (2.50 g) was separated by flash silica gel (100 g) chromatography providing alcohol **12** (0.39 g) and regenerated **4** (1.3 g). The alcohol **12** (0.39 g) was hydrogenated using P2 Ni (40 ml) as described before. Subsequent chromatographic purification afforded **13** (0.375 g, 95 %). ^1H NMR ($CDCl_3$) δ: 1.3-1.8 (18H, m), 2.01 (2H, dt, J = 6.7, 6.7 Hz, CH_2-C=), 3.40 (2H, m, CH_2[14]), 3.80 (2H, m, CH_2[6′]), 4.58 (1H, bs, CH[2′]), 5.34 (1H, bd, J = 11.9 Hz, H(C-5)), 5.37 (1H, dt, J = 11.0, 6.7 Hz, H(C-6)); ^{13}C NMR ($CDCl_3$) δ: 19.9, 25.7, 26.4, 27.4, 29.4, 2 × 29.6, 29.9, 30.0, 31.0, 62.6, 67.9, 99.1, 129.5, 130.6; IR cm^{-1}(percent transmittance): 3666 (99.67), 3012 (97.92), 2936 (85.70), 2367 (93.64), 2208 (98.25), 2109 (98.99), 1645 (97.84), 1458 (98.81), 1352 (99.10), 1250 (96.91), 1069 (94.80), 905 (98.73).

(5Z)-1-Iodo-14-(tetrahydropyran-2-yloxy)-[1,1,2,2,3,3,4,4,-$^2H_8$]tetradec-5-ene (14). An ice cold solution of **13** (0.37 g, 1.14 mmol) in dry pyridine (5 ml) was treated with *p*-toluensulfonyl chloride (0.23, 1.2 mmol) and kept in a freezer over night. The crystals that separated were removed by filtration, and the mother liquor was diluted with dry ether and the precipitate formed was filtered. The solution was washed with ice-cold H_2O (30 ml) followed by an aq.$CuSO_4$ (4 × 5 ml) to remove traces of pyridine. The solution was washed with H_2O (10 ml) and brine (5 ml) and dried over $MgSO_4$ and solvent was removed. The crude oil was dissolved in dry acetone (5 ml) and NaI (0.35 g, 2.3 mmol) was added.[15] After 18 h of stirring, the mixture was diluted with pentane (15 ml), the precipitated salts were filtered, and the crystals were washed with ether (2 × 15 ml). The solution was washed with aq. Na_2SO_3 solution (10 ml), H_2O (5 ml), and brine (5 ml), and concentrated. Subsequent flash silica gel (10 g) chromatography afforded the iodide **14** (0.37 g, 79%). ^1H NMR ($CDCl_3$) δ: 1.3-1.8 (18H, m), 2.01 (2H, dt, J = 7.0, 6.9 Hz, CH_2-C=), 3.40 (2H, m, CH_2[14]), 3.80 (2H, m, CH_2[6′]), 4.58 (1H, bs, CH[2′]), 5.31 (1H, bd, J = 11.3 Hz, H(C-5)), 5.39 (1H, dt, J = 11.0, 7.0 Hz, H(C-6)); ^{13}C NMR ($CDCl_3$) δ: 19.9, 25.7, 26.5, 27.5, 29.5, 2 × 29.7, 29.9, 30.0, 31.0, 62.6, 67.9, 99.1, 128.9, 131.0.

1-Bromo-[2,2,3,3,4,4,4-$^2H_7$]butane. A solution of [$^2H_7$]butyric acid (1g, 10.5 mmol) in dry THF (3 ml) was treated with a THF solution of borane (1M, 11 ml) maintaining the mixture under spontaneous reflux. The mixture was heated at 50 °C for 2 h, and cooled to room temperature. The solvent was evaporated and the resulting oil was dissolved in dry CH_2Cl_2 (10 ml). A saturated aq. solution of K_2CO_3 (0.5 ml) was added, the mixture was stirred for 3.5 h, and organic layer was dried with $MgSO_4$. The solvent was distilled, the product, [2,2,3,3,4,4,4-$^2H_7$]butan-1-ol, was dissolved in CH_2Cl_2 (4 ml) and treated with an ice-cold suspension of triphenylphosphine dibromide (11 mmol; prepared[21] from 2.9 g of Ph_3P and 0.57 ml Br_2) in CH_2Cl_2 (50 ml) for 0.5 h. The reaction mixture was washed with Na_2CO_3 solution (10%, 15 ml), $Na_2S_2O_3$ (saturated, 5 ml), H_2O (5 ml) and dried over $MgSO_4$. The solvent was removed and the residue was treated with pentane (10 ml) and left in a freezer. The crystals of triphenylphosphine oxide were separated, and the pentane solution was passed thorough a short silica gel (10 g) column and washed with pentane (15 ml). The pentane was removed, and the resulting yellowish oil was distilled (b.p. 150 °C) to give 1-bromo-[2,2,3,3,4,4,4-$^2H_7$]butane (0.54 g, 38 % from acid). ^{1}H NMR ($CDCl_3$) δ: 3.40 (2H, pentet, $^{1,3}J$ {H/D} = 1 Hz); ^{13}C NMR ($CDCl_3$) δ: 33.83 (C-1).

(9Z)-[11,11,12,12,13,13,14,14,16,16,17,17,18,18,18-$^2H_{15}$]Octadec-9-en-1-ol (15). CuCN (9 mg, 0.1 mmol) and LiCl (10 mg, 0.2 mmol) were added to a flask which was evacuated and purged with argon. The salts were dissolved in THF (0.3 ml) and a solution of iodide **14** (157 mg, 0.365 mmol) in THF (0.3 ml) was added. The mixture was cooled to -40 °C and the Grignard reagent in THF (0.5 ml, 0.5 mmol), prepared from 1-bromo-[2,2,3,3,4,4,4-$^2H_7$]butane, was added over a period of 0.5 h at -30 to -25 °C. The resulting brown-colored reaction mixture was stirred over night at 0 °C. The reaction was quenched with aq. NH_4Cl (2 ml), followed by a concentrated NH_4OH solution (0.5 ml). The product was removed with ether (3 × 5 ml), dried and concentrated. The residue was dissolved in methanol (10 ml) and *p*-toluensulfonic acid (10 mg) was added. After 2 h, powdered K_2CO_3 (0.5 g) was added and the mixture stirred for 10 min. The solids were filtered, and the filtrate was concentrated. Purification by silica gel chromatography eluting with petroleum ether/ether gave alcohol **15** as a colorless oil (90 mg, 88 % from **13**). ^{1}H NMR ($CDCl_3$) δ: 1.23 (2H, bs, CH_2[15]), 1.20-1.40 (10H, m, CH_2[3, 4, 5, 6, 7]), 1.55 (2H, m, CH_2[2]), 2.01 (2H, dt, J = 5.4, 6.7, CH_2[8]), 3.64 (2H, t, J = 6.7 Hz, CH_2[1]), 5.34 (2H, m, -CH=CH-); ^{13}C NMR ($CDCl_3$) δ: 25.9 (C-3), 27.4 (C-7), 29.1 (C-15), 29.5 (C-4*), 29.5 (C-8*), 29.6 (C-6*), 30.0 (C-5), 33.0 (C-2), 63.3 (C-1), 130.0 (C-9), 130.1 (C-10).

(9Z)-[11,11,12,12,13,13,14,14,16,16,17,17,18,18,18-$^2H_{15}$]Octadec-9-enoic acid (9). The alcohol **15** (83 mg) was dissolved in dry DMF (2.5 ml) and PDC (200 mg) was added. The resulting brown solution was stirred for 2 days. The acid **9** was purified as described above for **2**, yielding 33.4 mg (38 %) of a viscous oil. ^{1}H NMR ($CDCl_3$) δ: 1.23 (2H, bs, CH_2[15]), 1.38-1.30 (8H, m, CH_2[4, 5, 6, 7]), 1.63 (2H, tt, J = 4x7.0 Hz, CH_2[3]), 2.01 (2H, dt, J = 5.1, 6.8, 7.0, CH_2[8]), 2.35 (2H, t, J = 7.3, 7.6 Hz, CH_2[2]), 5.34 (2H, m, -CH=CH-); ^{13}C NMR ($CDCl_3$) δ: 24.9 (C-3), 27.4 (C-7), 29.0 (C-15), 29.2 (C-4*), 29.3 (C-6*), 29.4 (C-8*), 29.9 (C-5), 34.2 (C-2), 130.0 (C-9), 130.2 (C-10), 179.7 (C-1); EIMS (methyl ester, *m/z* (%): 312 (M^++1, 2), 311 (M^+, 8), 310 (M^+-1, 1), 280 (M^+-31, 36), 279 (M^+-32, 74), 237 (33), 195 (16), 123 (19), 110 (27), 98 (64), 87 (64), 84 (61), 74 (100), 59 (56), 55 (57), 41 (46); IR cm^{-1} (percent transmittance): 3576 (98.89), 3013 (99.31), 2935 (96.71), 2365 (99.95), 2361 (99.95), 2206 (98.95), 2106 (99.42), 1778 (97.30), 1458 (99.62), 1373 (99.66), 1122 (98.64).

(5Z)-11-Propyl-12-[13,13,14,14-$^2H_4$]azacyclotetradec-5-en-14-olide ([13,13,14,14-$^2H_4$]epilachnene) (**11**). This compound was synthesized according to the procedure described previously for eplilachnene.[18] The reaction scheme started from (2*S*)-2-amino-1-pentanol and used Br-CD_2-CD_2-OTBDMS as the deuteriated precursor. ^{1}H-NMR (500 MHz, C_6D_6) δ: 0.90-0.93 (3H, m), 1.05-1.40 (10H, m), 1.51 (1H,ddddd, J = 13.5, 8.6, 8.1, 5.9, 3.7), 1.64 (1H,ddddd, J = 13.4, 9.5, 6.8, 5.9, 3.4), 1.96 (2H, q, J = 7.1), 2.11 (1H, ddd, J = 16.4, 7.8, 3.4), 2.12-2.05 (1H, m), 2.17 (1 H, ddd, J = 16.4, 9.0, 3.4), 2.26-2.19 (1H, m), 2.33-2.28 (1H, m), 5.24-5.18 (1H, m), 5.42-5.36 (1H, m); LREIMS, see Table 1; HREIMS 271.2451 (calc. for $C_{16}H_{25}D_4N_1O_2$ 271.2449), 228.1901 (calc. for $C_{13}H_{18}D_4N_1O_2$ 228.1901), 174.1431 (calc. for $C_9H_{12}D_4N_1O_2$ 174.1432); IR cm^{-1}(percent transmittance): 3011 (96.90), 2936 (85.70), 2871 (94.20), 1750 (85.90).

Feeding Experiments. Young bean (*Phaseolus vulgaris*) plants, with the two primary leaves representing the only expanded foliage, were selected. After removal of one leaf from each plant, the underside of the remaining leaf was covered with fine nylon mesh to restrict larval feeding to the upper surface which was then coated as uniformly as possible with a single test compound. Oleic acid (labeled and control) was applied as ether solutions (1.5 mg in 40 μl ether/leaf). Similarly, stearic acid (labeled and control) was applied as aqueous suspensions (*ca.* 5 mg in 100 μl deionized water/leaf). Following solvent evaporation, a single, randomly selected, last instar *E. varivestis* larva was placed on each leaf to feed. There were two replicates per treatment. After 3.0 to 3.5 days, each larva had consumed most of the leaf surface and was then transferred to a new untreated plant to complete feeding. When prepupal anchorage was observed, larvae were placed in individual petri dishes to pupate. When each pupa was 3 days old, the defensive droplets on its secretory hairs were collected using a glass microcapillary. The secretion was then transferred to a 0.1 ml screw-capped vial containing 50 μl of dichloromethane in which the emptied microcapillary was rinsed. Samples were frozen (-20 ^{0}C) until analysis.

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