

Synthesis and Absolute Configuration of 2-(12'-Aminotridecyl)-pyrrolidine, a Defensive Alkaloid from the Mexican Bean Beetle, *Epilachna varivestis*¹

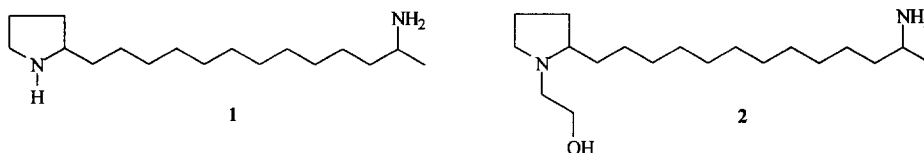
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Abstract: The synthesis of 2-(12'-aminotridecyl)-pyrrolidine (**1**), a defensive alkaloid recently isolated from the Mexican bean beetle, *Epilachna varivestis*, is described. The (2*S*,12'*R*) configuration is assigned to this alkaloid by comparing the ¹H NMR spectrum of its (*S*)-MTPA derivative with that of (*R*)- and (*S*)-MTPA [α -methoxy- α -(trifluoromethyl)phenylacetyl] derivatives of the synthetic sample. These results suggest that the seventeen carbon skeleton of **1** is acetate rather than proline derived. Copyright © 1996 Elsevier Science Ltd

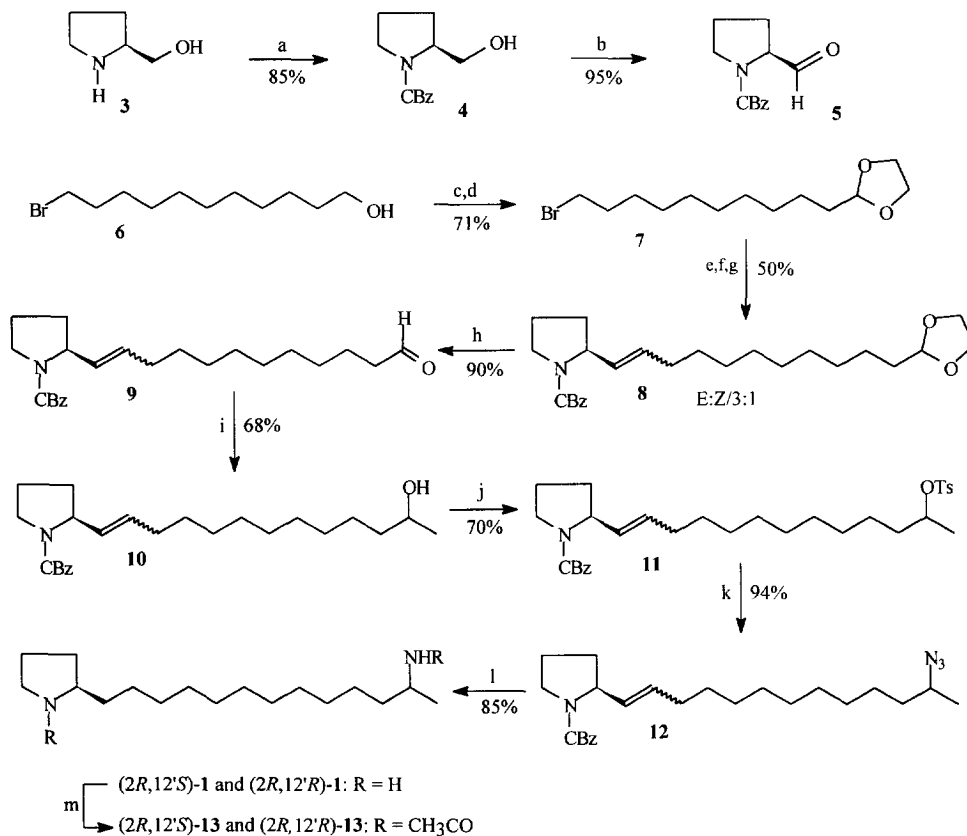
INTRODUCTION

Recently, we reported the characterization of two novel alkaloids, 2-(12'-aminotridecyl)-pyrrolidine (**1**) and its 1-(2-hydroxyethyl) derivative (**2**) from a whole body extract of the Mexican bean beetle, *Epilachna varivestis* (Coccinellidae).⁴ At the same time, an independent investigation of alkaloids present in eggs, larvae, pupae, and adults of *E. varivestis* showed, among other alkaloids, the presence of **2** in all four life stages, although this investigation did not report the occurrence of **1**.⁵ Our characterization of **1** relied mainly on its mass spectral and infrared absorption data as well as on chemical reactions, since the amount of **1** originally isolated was insufficient for NMR spectroscopy. Furthermore, the stereochemistry of the two chiral centers of both **1** and **2** remained unknown. In order to confirm the structure proposed for this alkaloid, to determine its relative and absolute configuration, and to get an adequate supply for biological testing, we have undertaken a synthesis of **1**. We now describe the synthesis of a diastereomeric mixture of 2-(12'-aminotridecyl)-pyrrolidines (**1**) as well as the determination of the absolute stereochemistry of the natural alkaloid **1** by ¹H NMR analysis of its MTPA [α -methoxy- α -(trifluoromethyl)phenylacetyl] derivative.



RESULTS AND DISCUSSION

As summarized in **Scheme 1**, (*S*)-*N*-CBz-pyrrolidine-2-carboxaldehyde **5** was prepared in good yield from the commercially available L-prolinol (**3**) in two steps: protection of the amino group with benzyl chloroformate⁶, and Swern oxidation⁷ of the hydroxyl group of **4** gave the corresponding aldehyde **5**. The bromide **7** was obtained from 11-bromo-1-undecanol (**6**) in 71% overall yield in two steps: oxidation with pyridinium chlorochromate (PCC)⁸ followed by protection of the resulting aldehyde by reaction with ethylene glycol.



Scheme 1. (a) ClCOOCH₂Ph/NaOH, THF, 25 °C, 20 h; (b) (COCl)₂, DMSO, TEA, -78 °C — 25 °C, 2 h; (c) PCC, CH₂Cl₂, 25 °C; (d) HOCH₂CH₂OH, *p*-TsOH, C₆H₆, reflux, 4 h; (e) Ph₃P, CH₃CN, reflux, 60 h; (f) *n*-BuLi, THF, -78 °C — 25 °C, 12 h; (g) **5**, THF, -78 °C — 25 °C, 12 h; (h) 1M HCl, acetone, 25 °C, 6 h; (i) CH₃MgBr, ether, -30 °C — 25 °C; (j) TsCl/pyridine, 25 °C, 20 h; (k) NaN₃, DMF, 80—90 °C, 3 h; (l) H₂/Pd, THF 25 °C, 4 h; (m) (CH₃CO)₂O, pyridine, 25 °C, 2 h.

Treatment of **7** with triphenylphosphine in refluxing CH₃CN gave the corresponding phosphonium salt which was further treated with *n*-BuLi to generate *in situ* the desired ylide.⁹ Wittig coupling of this ylide with aldehyde **5** afforded a mixture of olefins (**8**, *E:Z ca.* 3:1) in 50% overall yield.⁹ Deprotection of **8** by treatment with hydrochloric acid provided aldehyde **9** (yield: 90%) which was then subjected to a nucleophilic addition reaction with CH₃MgBr to afford alcohol **10** in 68% yield. Tosylation of secondary alcohol **10** (yield: 70%), followed by treatment with NaN₃ in DMF, led to azide **12** in 94% yield.¹⁰ Reduction of the azide group, hydrogenation of the double bond, and deprotection of the amino group were accomplished simultaneously by reaction with H₂/Pd on activated carbon,¹⁰ providing a diastereomeric mixture of (2*R*,12'*S*)-**1** and (2*R*,12'*R*)-**1** in 85% yield.

Retention time and spectroscopic data of the unresolved synthetic mixture of (2*R*,12'*S*)-**1** and (2*R*,12'*R*)-**1**, obtained by capillary gas chromatography, GC-MS, and GC-IR on the achiral stationary phase DB-5, were indistinguishable from those of the natural alkaloid **1**. Moreover, GC-MS and GC-IR properties of the *bis*-acetyl derivatives (**13**), derived from both the natural alkaloid and the synthetic material, were also indistinguishable.⁴ In addition, ¹H and ¹³C NMR spectral data of the synthetic sample were congruent with those of the natural product, which we re-isolated from 360 freshly collected *E. varivestis* adults. The proposed structure for this defensive alkaloid, 2-(12'-aminotridecyl)-pyrrolidine (**1**), could therefore be confirmed. However, the stereochemistry remained to be addressed, since we were unable to establish the absolute configuration of the natural alkaloid (**1**) using X-ray crystallography, and the diastereomers of **1** show identical ¹H and ¹³C NMR and infrared spectral data. In addition, neither diastereomeric mixture could be resolved by gas chromatography, as a consequence of the large spacial separation between the two asymmetric centers.

The apparent lack of interaction between the two chiral centers of **1** suggested that the absolute configuration of each center could probably be determined independently by attaching an additional chiral center of known configuration onto both the primary and secondary amino groups. The synthetic diastereomeric mixture of the alkaloid **1** was acylated with (*R*)- α -methoxy- α -(trifluoromethyl)phenylacetyl chloride to give a mixture of two epimeric diamides, (α *S*,2*R*,12'*S*, α *S*)-**14** and (α *S*,2*R*,12'*R*, α *S*)-**14**.¹² As illustrated in **Fig. 1 (A)**, ¹H NMR spectral analysis of the epimeric mixture showed clearly that the doublets corresponding to the terminal methyl group (δ 1.12 and 1.18, *J* = 6.4 Hz), and the doublets arising from the NH group attached to C-12' (δ 6.48 and 6.54, *J* = 8.7 Hz) were well resolved, while the signals for the pyrrolidine ring protons at C-5 and C-2 (δ 2.82 and 3.18, and δ 4.19) showed no apparent differences for the two epimers. The chemical shift difference observed for the two methyl doublets of (α *S*,2*R*,12'*S*, α *S*)-**14** and (α *S*,2*R*,12'*R*, α *S*)-**14** can be explained using extended Newman projections (**Fig. 2**), as proposed by Mosher *et al.*¹² The upfield doublet (δ 1.12, *J* = 6.4 Hz) was assigned to the 12'*S*-epimer, and the downfield doublet (δ 1.18, *J* = 6.4 Hz) to the 12'*R*-epimer, based on the anisotropic diamagnetic shielding effect of the benzene ring in the (*S*)-MTPA moiety.¹³

The ¹H NMR data of the diamide (**14**) derived from the natural alkaloid with (*R*)- α -methoxy- α -(trifluoromethyl)phenylacetyl chloride [see **Fig. 1 (B)**] are clearly different from those of the derivatives of the

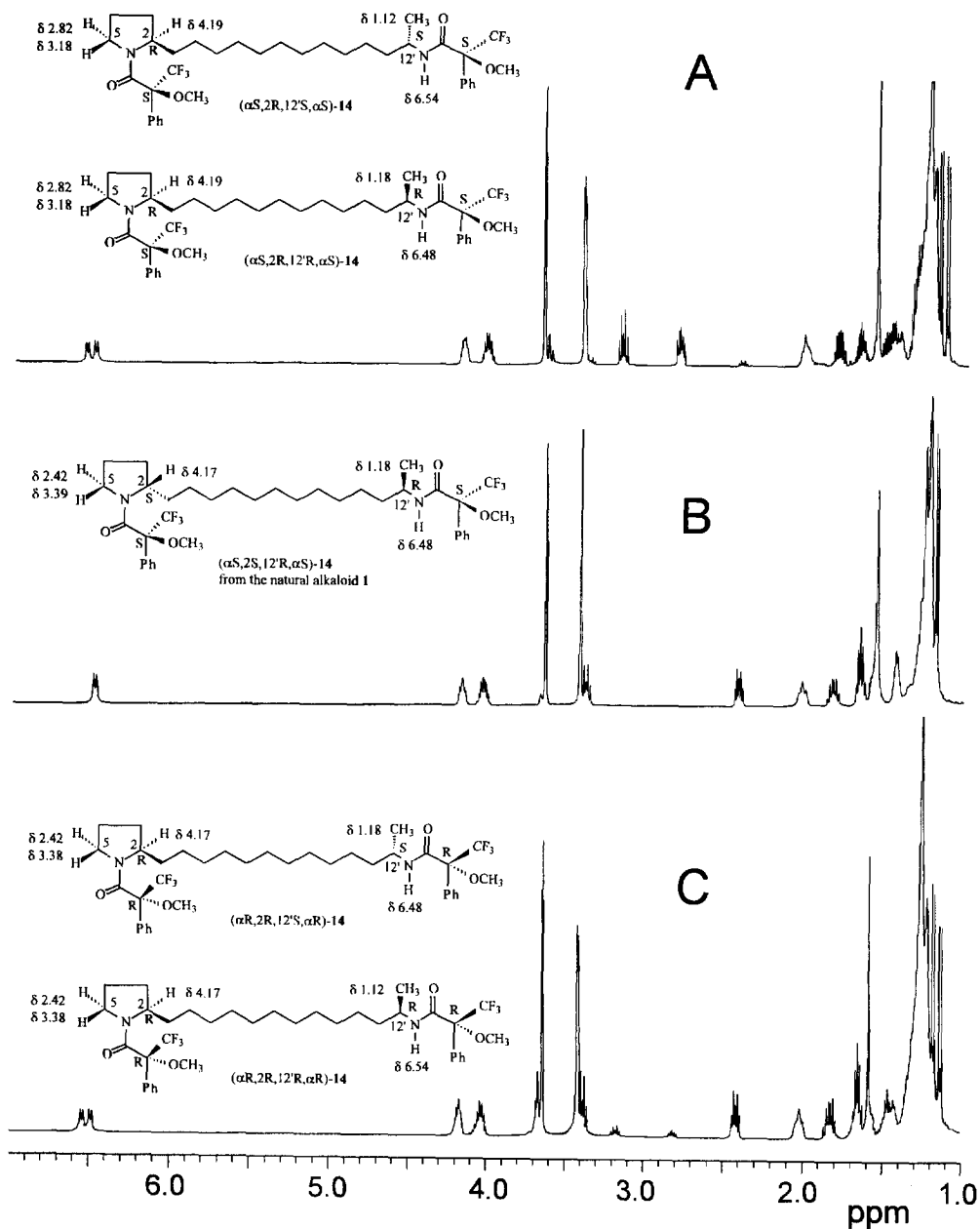


Figure 1. ^1H NMR data of MTPA derivatives of synthetic and natural samples of 1.

synthetic alkaloids, ($\alpha S, 2R, 12'R, \alpha S$)-**14** and ($\alpha S, 2R, 12'S, \alpha S$)-**14** [Fig. 1 (A)]. A close examination of the ^1H NMR data of the derivatives of the synthetic alkaloids [Fig. 1 (A)] revealed weak signals at δ 2.42 and 3.39 which in fact are due to the presence of *ca.* 5-10% of the C-2 epimer derivative. These chemical shifts are consistent with those of the diamide derived from the natural alkaloid [Fig. 1 (B)]. Accordingly, the configuration at C-2 of the natural alkaloid **1** must be *S* rather than *R*. Thus, the natural alkaloid's stereochemistry must be either (*2S, 12'S*)-**1** or (*2S, 12'R*)-**1**. Since the diamide derived from the natural alkaloid shows a chemical shift for its methyl group (δ 1.18, $J = 6.4$ Hz) identical to that of ($\alpha S, 2R, 12'R, \alpha S$)-**14**, we can assign the absolute configuration of the natural alkaloid as (*2S, 12'R*)-**1**.



Figure 2. Extended Newman projections of C-12' epimers of (*S*)-MTPA diamide.

To support this assignment based on the hypothesis that the two asymmetric centers in alkaloid **1** have no apparent mutual effects either on ^1H NMR chemical shifts or coupling patterns, we prepared a mixture of the other pair of diamide epimers, ($\alpha R, 2R, 12'R, \alpha R$)-**14** and ($\alpha R, 2R, 12'S, \alpha R$)-**14**, from the mixture of synthetic diastereomers of **1** with (*S*)-Mosher acid chloride. The ^1H NMR signals of either ($\alpha R, 2R, 12'R, \alpha R$)-**14** or ($\alpha R, 2R, 12'S, \alpha R$)-**14** for protons at C-5 and C-2 positions (δ 2.42, 3.38, and 4.17) matched with those of the (*S*)-MTPA derivative of the natural alkaloid, as shown in Fig. 1 (C), indicating that the (*S*)-MTPA derivative of the natural alkaloid bears the ($\alpha S, 2S$)-configuration. Furthermore, the methyl doublet at δ 1.18, resulting from the epimer ($\alpha R, 2R, 12'S, \alpha R$)-**14** as predicted by the Mosher method, showed a chemical shift and coupling pattern identical to that of the (*S*)-MTPA derivative of the natural alkaloid **1**. These results revealed that the (*S*)-MTPA derivative of the natural alkaloid **1** must have the ($\alpha S, 2S, 12'R, \alpha S$)-configuration, which corresponds to that of the enantiomer of ($\alpha R, 2R, 12'S, \alpha R$)-**14**. Thus, the absolute stereochemistry of natural **1** was again assigned to be (*2S, 12'R*)-**1**. This conclusion was subsequently confirmed by stereospecific synthesis of (*2S, 12'R*)-**1** and its diastereomer, (*2S, 12'S*)-**1**.¹⁴

Although we initially considered that *E. varivestis* might utilize the naturally occurring L-proline as a precursor for the biosynthesis of **1**, and consequently chose L-prolinol as our chiral synthetic starting material, our results now suggest that **1** is most likely acetate-derived. The expectation that 1-(2-hydroxyethyl)-2-(12'-aminotridecyl)-pyrrolidine (**2**) has the same absolute stereochemistry as **1** was confirmed by the synthesis of (*2S, 12'R*)-**2** starting from D-prolinol, details of which, along with the enantiospecific synthesis of (*2S, 12'R*)-**1**, will be reported in another publication.¹⁴

EXPERIMENTAL

General experimental procedures. Low-resolution EI-mass and gas-phase infrared spectra were obtained using an HP 5890 gas chromatograph linked to an HP mass selective detector (MSD) and an HP 5965A IR detector. Gas chromatographic analyses were performed using a 25 m x 0.32 mm fused-silica column coated with DB-5. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained using a Varian XL-400 spectrometer unless otherwise noted. Chemical shifts are given in ppm relative to ^1H NMR peak of CHCl_3 (residue in CDCl_3) at 7.26 and ^{13}C NMR middle peak of CDCl_3 at 77.0 ppm, respectively. Flash column chromatography was performed over silica gel (60 μm , EM Science, Gibbstown, NJ) and monitored by TLC utilizing precoated 0.25 mm silica-gel plates with Fluorescent indicator. High-resolution EI mass spectra (HREI) were recorded using a Finnigan-MAT 731 instrument. For high-resolution CI mass spectra (HRCI), a VG 70-VSE instrument was used.

(2S)-1-Benzoyloxycarbonyl-2-(hydroxymethyl)-pyrrolidine (4). To a solution of L-prolinol (3) (670 mg, 6.6 mmol) in THF (25 ml) were added aqueous 2 M NaOH (16 ml) and benzyl chloroformate (2.47 g, 14.5 mmol). After stirring at room temperature for 20 h, the organic phase was separated and the aqueous phase was extracted with EtOAc. The combined organic phase was washed with water and dried over Na_2SO_4 . After removal of the solvent, the residue was purified by column chromatography over silica gel (40-60% EtOAc in hexane) to afford **4** (1.33 g, 85%) as a colorless oil. HREIMS ($R = 10,000$) 235.12092 (M^+ , calcd for $\text{C}_{13}\text{H}_{17}\text{N}_1\text{O}_3$: 235.12084); LREIMS m/z (%) 235 (M^+ , 1), 205 (6), 204 (44), 160 (29), 92 (8), 91 (100), 65 (8); ^1H NMR δ 1.58 (1H, m), 1.84 (2H, m), 2.02 (1H, m), 2.20 (br, OH), 3.40 (1H, dt, $J = 10.5$, 6.6 Hz), 3.55 (1H, dt, $J = 10.5$, 7.0 Hz), 3.63 (2H, m), 4.01 (1H, br), 5.14 (2H, d, $J = 1.8$ Hz), 7.30-7.37 (5H, m); IR 3500, 3039, 2974, 2890, 1801, 1724, 1404, 1353, 1189, 1097 cm^{-1} .

(2S)-1-Benzoyloxycarbonylpyrrolidine-2-carboxaldehyde (5). To a solution of oxalyl chloride in CH_2Cl_2 (2M, 3.4 ml) was added dropwise a solution of DMSO (0.65 ml, 9.0 mmol) in CH_2Cl_2 (7 ml) at -78°C over a period of 30 min. After stirring for 15 min, a solution of N-CBz-prolinol (**4**) (1.05 g, 4.47 mmol) in CH_2Cl_2 (25 ml) was added. After stirring for 30 min, triethylamine (1.8 ml, 13 mmol) was added to the mixture. The reaction mixture was allowed to warm to room temperature and quenched with water. The organic phase was washed with 20% KHSO_4 , saturated NaHCO_3 , and brine, and dried over Na_2SO_4 . Evaporation of the solvent gave aldehyde **5** (990 mg, 95%) as a slightly yellow oil. HREIMS ($R = 10,000$) 233.10516 (M^+ , calcd for $\text{C}_{13}\text{H}_{15}\text{N}_1\text{O}_3$: 233.10519); LREIMS m/z (%) 233 (M^+ , 0.2), 205 (8), 204 (59) 160 (24), 92 (8), 91 (100), 65 (10); ^1H NMR (rotamers/1:1) δ 1.80-1.99 (2H, m), 2.01-2.18 (2H, m), 3.52-3.57 (2H, m), 4.21 and 4.30 [1H, (dt, $J = 6.5$, 2.2 Hz, and dt, $J = 6.5$, 1.3 Hz)], 5.13 and 5.17 [2H, (s, and d, $J = 4.3$ Hz)], 7.29-7.38 (5H, m), 9.47 and 9.58 [1H, (d, $J = 2.4$ Hz and d, $J = 1.6$ Hz)]; ^{13}C NMR δ (23.7, 24.5), (26.6, 27.8), (46.7, 47.3), (67.2, 67.3), 76.8, 128.0, 128.1, 128.5, (136.2, 136.4), (154.5, 155.3), 200.0; IR 2980, 2892, 2802, 2364, 1733, 1454, 1402, 1347, 1267, 1181, 1100, 974 cm^{-1} .

1-Bromo-11-ethylenedioxyundecane (7). To a solution of pyridinium chlorochromate (4.86 g, 22.5 mmol) in CH_2Cl_2 (25 ml) was added dropwise a solution of 11-bromo-1-undecanol (**6**) (3.77 g, 15 mmol) in CH_2Cl_2 (15 ml). After the reaction mixture was stirred at room temperature for 2 h, anhydrous ether (*ca.* 15 ml) was added. The precipitate was removed by filtration of the reaction mixture through a thin layer of anhydrous MgSO_4 . The filtrate was concentrated to give the crude aldehyde which was immediately used in the next reaction. A solution of the crude aldehyde (4.30 g), ethylene glycol (1.1 ml, 19.5 mmol) and *p*-toluenesulfonic acid (40 mg) in dry benzene (60 ml) was refluxed for 4 h. After cooling and addition of ether (100 ml), the solution was washed successively with solutions of saturated NaHCO_3 (30 ml), brine (30 ml), and water (40 ml). The solution was dried and concentrated to afford a residue which was purified by column chromatography over silica gel (10% ether in hexane) to give acetal **7** (3.10 g, 71% in two steps) as a pale yellow oil. HRCIMS ($R = 5,000$) 293.1103 (calcd for $\text{C}_{13}\text{H}_{26}\text{O}_2\text{Br}$, 293.1116); LRCIMS m/z (%) 295 (44), 293 (53), 73 (100); LREIMS m/z (%) 294 and 292 (M^+ , 0.2), 293 and 291 ($\text{M}^+ - 1$, 1), 99 (1), 95 (1), 73 (100); ^1H NMR δ 1.27-1.50 (14H, m), 1.64 (2H, dt, $J = 5.2, 7.0$ Hz), 1.84 (2H, five peaks, $J = 7.4$ Hz), 3.40 (2H, t, $J = 6.8$ Hz), 3.84 (2H, s), 3.97 (2H, s), 4.83 (1H, t, $J = 4.9$ Hz); IR 2935, 2867, 1457, 1401, 1257, 1139, 1047, 942 cm^{-1} .

(2S)-1-Benzoyloxycarbonyl-2-(12'-ethylenedioxy-1'-dodeceny)-pyrrolidine (8). To a solution of bromide **7** (2.93 g, 10 mmol) in dry CH_3CN (16 ml) was added triphenylphosphine (2.63 g, 10 mmol). The mixture was stirred and refluxed for 60 h before allowing it to cool to room temperature. The solvent was evaporated and the residue was washed with ether (4 x 20 ml) and then dried under vacuum to give the crude phosphonium bromide (5.3 g, 95%). This was used directly in the next step without further purification.

To a stirred solution of the phosphonium bromide (3.00g, 5.5 mmol) in THF (45 ml) was added dropwise *n*-BuLi (1.6 M in hexane, 3.8 ml) at -78°C . The reaction mixture was gradually warmed to room temperature and allowed to stand for 30 min. After being cooled to -78°C , a solution of **5** (0.98 g, 4.2 mmol) in THF (45 ml) was added dropwise to the solution. The resulting mixture was gradually warmed to room temperature and then stirred overnight. The reaction mixture was quenched with ice/water and extracted with ether. The organic phase was dried and concentrated to give a residue which was purified by flash chromatography over silica gel (20-30% EtOAc in hexane) to give product **8** (960 mg, 53%) as an oil. HRCIMS ($R = 5,000$) 430.2950 (calcd for $\text{C}_{26}\text{H}_{40}\text{O}_4\text{N}$, 430.2957); LREIMS m/z (%) 429 (M^+ , 0.2), 338 (5), 294 (35), 276 (1), 265 (2), 250 (3), 204 (5), 186 (4), 160 (8), 114 (11), 91 (100), 73 (78); ^1H NMR δ 1.08-1.50 (16H, m), 1.80-2.11(4H, m), 3.48 (2H, t, $J = 6.7$ Hz), 3.84 (2H, s), 3.96 (H, s), 4.59 (1H, br s), 4.83 (1H, t, $J = 5.1$ Hz), 5.10 (2H, s), 5.28-5.40 (2H, m), 7.28-7.35 (5H, m); ^{13}C NMR δ 24.1, 27.3, 29.1-29.7 (m), 33.9, 64.8, 66.5, 104.7, 126.9-130.0 (m), 137.1, 154.9; IR 2933, 2362, 1724, 1401, 1350, 1100 cm^{-1} .

(2S)-1-Benzoyloxycarbonyl-2-(11'-oxo-1'-dodeceny)-pyrrolidine (9). A solution of the Wittig product **8** (860 mg, 2.0 mmol) and 1 M HCl (10 ml) in acetone (45 ml) was stirred at room temperature for 8 h. After evaporation of the solvent, water (1 ml) was added to the above solution. The solution was extracted with ether and the combined extract was washed with saturated NaHCO_3 solution and brine successively and dried over MgSO_4 . Evaporation of the solvent gave crude aldehyde **9** (695 mg, 90%) as a slightly yellow oil. This was directly used in the next reaction. HRCIMS ($R = 5,000$) 386.2699 (calcd for $\text{C}_{24}\text{H}_{36}\text{O}_3\text{N}$

386.2695); LREIMS m/z (%) 355 (M^+ -30, 2), 342 (2), 294 (12), 250 (100), 222 (5), 204 (7), 186 (9), 160 (5), 114 (9), 91 (61); ^1H NMR δ 1.14-1.70 (16H, m), 1.80-2.20 (4H, m), 2.41 (2H, dt, $J = 1.8, 7.3\text{Hz}$), 3.46 (2H, m), 4.59 (1H, br s), 5.09 (2H, s), 5.24-5.40 (2H, m), 7.27-7.32 (5H, m), 9.76 (1H, t, $J = 1.8\text{ Hz}$); ^{13}C NMR δ 22.1, 24.1, 27.3, 29.1-29.7 (m), 33.9, 43.9, 46.8, (54.3, 54.7), (64.8, 65.4), 127.0-137.1 (m), 202.9; IR 2934, 2709, 2356, 1724, 1453, 1401, 1350, 1272, 1180, 1098 cm^{-1} .

(2S,12'SR)-1-Benzoyloxycarbonyl-2-(12'-hydroxy-1'-trideceny)-pyrrolidine (10). To a solution (1.5 ml) of 1 M CH_3MgBr in ether was added dropwise a solution of aldehyde **9** (145 mg, 0.377 mmol) in ether (1 ml) at -30°C to -25°C . The resulting mixture was gradually warmed to about 0°C and then quenched with ice/water. The solution was extracted with CH_2Cl_2 (3 x 10 ml). The combined organic phase was washed with water and dried over MgSO_4 . After evaporation of the solvent, the residue was purified by column chromatography over silica gel (20-30 % EtOAc in hexane) to afford alcohol **10** (102 mg, 68%) as an oil. HRCIMS ($R = 5,000$) 402.2992 (calcd for $\text{C}_{25}\text{H}_{40}\text{O}_3\text{N}_1$ 402.3008); LREIMS m/z (%) 310 (M^+ -91, 1), 266 (18), 248 (7), 186 (4), 160 (7), 114 (14), 91 (100), 45 (10); ^1H NMR δ 1.10-2.20 (22H, m), 1.17 (3H, d, $J = 7.0\text{ Hz}$), 3.46 (2H, m), 3.76 (1H, m), 4.59 (1H, br, s), 5.09 (2H, s), 5.24-5.40 (2H, m), 7.27-7.32 (5H, m); IR 2933, 2363, 1724, 1452, 1401, 1350, 1178, 1098 cm^{-1} .

(2S,12'SR)-1-Benzoyloxycarbonyl-2-[12'-(*p*-toluenesulfonyl)oxy-1'-trideceny]-pyrrolidine (11). Alcohol **10** (196 mg, 0.49 mmol) in anhydrous pyridine (2.5 ml) was treated with *p*-toluenesulfonyl chloride (196 mg, 0.98 mmol) at room temperature for 5 h. Additional *p*-toluenesulfonyl chloride (80 mg, 0.40 mmol) was added to the mixture. The reaction mixture was stirred at room temperature for 24 h, and then diluted with EtOAc (30 ml), washed with saturated CuSO_4 solution (3 x 1 ml) and brine, and dried over MgSO_4 . After removal of the solvent, the residue was purified by column chromatography over silica gel (10-25 % EtOAc in hexane) to give tosylate **11** (190 mg, 70%) as a colorless oil. HRCIMS ($R = 5,000$) 456.3084 (calcd for $\text{C}_{32}\text{H}_{46}\text{O}_5\text{N}_1\text{S}_1$ 430.2957); LREIMS m/z (%) 446 (1), 383 (3), 292 (10), 248 (27), 114 (24), 91 (100); ^1H NMR δ 1.05-2.20 (22H, m), 1.30 (3H, d, $J = 7.0\text{ Hz}$), 2.43 (3H, s), 3.46 (2H, t, $J = 6.2\text{ Hz}$), 4.60 (2H, six peaks, $J = 6.1, 6.4\text{ Hz}$), 5.09 (2H, s), 5.24-5.40 (2H, m), 7.33 (7H, s), 7.79 (2H, d, $J = 8.2\text{ Hz}$).

(2R,12'SR)-2-(12'-Aminotridecyl)-pyrrolidine (1). Tosylate **11** (70 mg, 0.125 mmol) in DMF (4 ml) was treated with NaN_3 (20 mg, 0.3 mmol in 0.4 ml of H_2O). The reaction solution was heated to $80\text{-}90^\circ\text{C}$ for 3 h. After being cooled, water (1 ml) was added to the solution which was then extracted with CH_2Cl_2 (3 x). The combined organic phase was dried and evaporated to give azide **12** (50 mg, 94 %) as a pale yellow oil. This crude product was directly used in the next reaction. ^1H NMR δ 1.10-2.20 (25H, m), 3.30-3.52 (3H, m), 4.60 (1H, br, s), 5.09 (2H, s), 5.24-5.40 (2H, m), 7.28-7.32 (5H, m).

The azide (30 mg, 0.07 mmol) in THF (3 ml) was stirred in the presence of 10% Pd-C (*ca.* 6 mg) and H_2 under atmospheric pressure and at room temperature for 6 h. Filtration of the catalyst and evaporation of the solvent gave a residue which was further purified by column chromatography over silica gel (CH_2Cl_2 :MeOH: NH_4OH ; 7:3:0.3) to afford the final product **1** (16 mg, 85%). GC, GC-MS, GC-IR, ^1H and ^{13}C NMR analyses showed synthetic **1** to be indistinguishable in all respects from the natural alkaloid. HRCIMS ($R = 5,000$) 267.2791 (calcd for $\text{C}_{17}\text{H}_{35}\text{N}_2$ 267.2800); LRCIMS m/z (%) 269 (M^+ +1, 84), 267 (100).

Extraction, isolation and purification of the natural alkaloids. Freshly collected *Epilachna varivestis* beetles (360 specimens) were immersed in 2% sulfuric acid in methanol (45 ml), crushed, and left for 2 h at room temperature. The supernatant was removed and the residue was re-extracted with the same acidic solvent (2 x 15 ml). The combined extract was concentrated to ca. 2 ml and diluted with water (10 ml). The solution was extracted with ether (6 x 10 ml). The alkaloids were released from the aqueous solution by adding concentrated KOH solution. The alkaloids were extracted with CH₂Cl₂ (3 x 10 ml) and the combined extract was washed with water and concentrated to give a yellow oil (ca. 5-10 mg). The crude alkaloids were subjected to flash chromatography over silica gel (60 μm, EM Science, Gibbstown, NJ). The column (25 cm x 3 mm, silica gel: 0.6 g) was eluted with CH₂Cl₂:MeOH:NH₄OH (9:1:0.05 for the first 10 fractions, 8:2:0.05 for the second 10 fractions, 7:3:0.05 for the third 10 fractions, and 6:4:0.05 for the fourth 10 fractions. The fractions) (ca. 1 ml/fraction) were monitored by TLC using Dragendorff reagent as indicator. Among other alkaloids, alkaloid **2** (2.6 mg, [α]_D²² +38.8°, c 0.18, CDCl₃) (fraction 12-16) and alkaloid **1** (1.5 mg, [α]_D²² +9.3°, c 0.15, CDCl₃) (fraction 37-39) were obtained. For alkaloid **1**: MS *m/z* (%) 268 (M⁺, 0.2), 267 (M⁺-1, 0.3), 254 (0.3), 253 (2), 224 (1), 200 (1), 199 (1), 182 (1), 168 (1), 154 (1), 126 (1), 98 (2), 96 (3), 84 (2), 83 (3), 70 (100), 56 (7), 55 (5), 44 (52), 43 (8), 42 (3), 41 (7); ¹H NMR (500 MHz) (Varian Unity 500 spectrometer) δ 1.03 (3H, d, *J* = 6.4 Hz), 1.2-1.5 (22H, m), 1.71 (2H, m), 1.87 (2H, m), 2.80-2.86 (2H, m), 2.94 (1H, br, t, *J* = 6.8 Hz), 3.01 (1H, ddd, *J* = 10.5, 7.5, 5.5 Hz); ¹³C NMR δ 24.0, 25.2, 26.5, 27.5, 29.6-29.8 (m), 31.8, 36.1, 40.3 46.3, 46.9, 59.5; IR 2932, 2864, 1622, 1459, 1362, 790 cm⁻¹.

Diacetyl derivatives of 2-(12'-aminotridecyl)-pyrrolidine (13). Small amounts of diacetyl derivative **13** of both the synthetic sample and the natural alkaloid **1** were prepared according to the method described previously.⁴ The GC-MS and GC-IR data of these diacetyl derivatives were indistinguishable.

MTPA diamides of 2-(12'-aminotridecyl)-pyrrolidine (14). To a CH₂Cl₂ (100 μl) solution of natural alkaloid **1** (ca. 1 mg) was injected dry pyridine (5 μL). After the solution was cooled to -20 °C, (*R*)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (5 μL) was injected. After 20 h at the temperature, the reaction mixture was subjected to flash chromatography over silica gel, using ether/hexane (1:1) as eluent. All fractions containing product were combined and concentrated to give ca. 1 mg of MTPA diamide (α*S*,2*S*,12'*R*,α*S*)-**14** for ¹H NMR analysis. ¹H NMR (500 MHz) (Varian Unity 500 spectrometer) δ 1.18 (3H, d, *J* = 6.4 Hz), 1.22-1.32 (19H, m), 1.43 (2H, dt, *J* = 1.3, 4.9 Hz), 1.55-1.60 (1H, m), 1.66 (2H, dt, *J* = 6.5, 6.5 Hz), 1.83 (1H, dq, *J* = 12.2, 7.8 Hz), 2.02 (1H, t, br), 2.42 (1H, ddd, *J* = 11.5, 6.4, 6.4 Hz), 3.39 (1H, ddd, *J* = 11.5, 7.3, 6.8 Hz), 3.42 (3H, d, *J* = 1.4 Hz), 3.64 (3H, s), 4.04 (1H, dtq, *J* = 8.7, 6.8, 6.4 Hz), 4.17 (1H, m), 6.48 (1H, d, *J* = 8.7 Hz), 7.35-7.40 (3H, m), 7.50-7.54 (2H, m).

In the same manner, the diastereomeric mixture of (α*S*,2*R*,12'*R*,α*S*)-**14** and (α*S*,2*R*,12'*S*,α*S*)-**14** was obtained from the synthetic diastereomeric mixture of (2*R*,12'*R*)-**1** and (2*R*,12'*S*)-**1** with (*R*)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride. ¹H NMR (500 MHz) (Varian Unity 500 spectrometer) δ 1.12 and 1.18 (3H, 2d, *J* = 6.4 Hz), 1.22-1.40 (19H, m), 1.40-1.60 (3H, m), 1.66 (2H, m, *J* = 6.5 Hz), 1.81 (1H, dq, *J* = 12.2, 7.0 Hz), 2.01 (1H, br), 2.82 (1H, ddd, *J* = 10.8, 5.6, 5.8 Hz), 3.18 (1H, ddd, *J* = 11.6, 7.3, 7.0 Hz), 3.42 (3H, m), 3.66 (3H, d, *J* = 1.8 Hz), 4.04 (1H, m), 4.19 (1H, m), 6.48 and 6.54 (1H, 2d, *J* = 8.7 Hz), 7.35-7.40 (3H, m), 7.50-7.54 (2H, m).

Similarly, the diastereomeric mixture of ($\alpha R, 2R, 12'R, \alpha R$)-**14** and ($\alpha R, 2R, 12'S, \alpha R$)-**14** was obtained from the synthetic diastereomeric mixture of ($2R, 12'R$)-**1** and ($2R, 12'S$)-**1** with (*S*)-(+)- α -methoxy- α -(trifluoromethyl)phenylacetyl chloride. $^1\text{H NMR}$ (500 MHz) (Varian Unity 500 spectrometer) δ 1.12 and 1.18 (3H, 2d, $J = 6.4$ Hz), 1.22-1.40 (19H, m), 1.40-1.60 (3H, m), 1.66 (2H, dt, $J = 6.5, 6.5$ Hz), 1.81 (1H, dq, $J = 12.2, 7.8$ Hz), 2.02 (1H, br), 2.42 (1H, ddd, $J = 11.5, 6.4, 6.4$ Hz), 3.38 (1H, ddd, $J = 11.5, 7.3, 6.8$ Hz), 3.41 and 3.42 (3H, 2d, $J = 1.0, 1.4$ Hz), 3.64 (3H, d, $J = 1.4$ Hz), 4.04 (1H, m), 4.18 (1H, m), 6.48 and 6.54 (1H, 2d, $J = 8.7$ Hz), 7.35-7.40 (3H, m), 7.50-7.54 (2H, m).

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