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Synthesis and Absolute Configuration of Two Defensive Alkaloids from the Mexican Bean Beetle, *Epilachna varivestis*[1]

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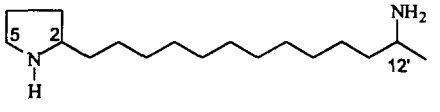
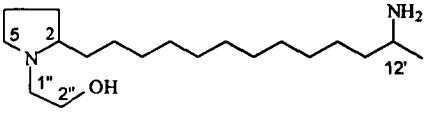
Abstract: Syntheses of (2*S*,12′*R*)-2-(12′-aminotridecyl)-pyrrolidine (**1**) and (2*S*,12′*R*)-1-(2″-hydroxyethyl)-2-(12′-aminotridecyl)-pyrrolidine (**2**), two defensive alkaloids recently isolated from the Mexican bean beetle, *Epilachna varivestis*, are described. By a comparison of ^{1}H NMR data of MTPA derivatives of natural alkaloid **2** with those of the synthetic standard, we confirm the (2*S*,12′*R*) configuration previously suggested for this alkaloid. Further support of these assignments was provided by the synthesis and ^{1}H NMR investigation of (2*S*,12′*S*)-**1**, (2*S*,12′*S*)-**2**, and their MTPA derivatives. © 1997 Published by Elsevier Science Ltd.

The hemolymph of ladybird beetles (Coleoptera, Coccinellidae) often contains a highly diverse set of alkaloids which protect these insects from predators.[2] Recently, our group characterized two alkaloids, 2-(12′-aminotridecyl)-pyrrolidine (**1**) and 1-(2″-hydroxyethyl)- 2-(12′-aminotridecyl)-pyrrolidine (**2**), from the Mexican bean beetle, *Epilachna varivestis*.[3] Proksch *et al.* also showed the presence of **2**, along with other alkaloids, in all four life stages of this beetle.[4] To determine the stereochemistry of **1**, we synthesized a diastereomeric mixture of (2*R*,12′*R*)-**1** and (2*R*,12′*S*)-**1** and assigned the (2*S*,12′*R*) configuration to the natural alkaloid **1** by a comparison of ^{1}H NMR data of the MTPA diamide of the natural alkaloid **1** with those of the synthetic material.[5] Since **2** is structurally very similar to **1**, we anticipated that **2** should have the same stereochemistry. We now describe syntheses of two pairs of diastereomers, (2*S*,12′*R*)-**1** and (2*S*,12′*S*)-**1**, (2*S*,12′*R*)-**2** and (2*S*,12′*S*)-**2**, and confirm the (2*S*,12′*R*) configuration for both natural alkaloids by ^{1}H NMR comparison of their MTPA derivatives with those of these synthetic samples.



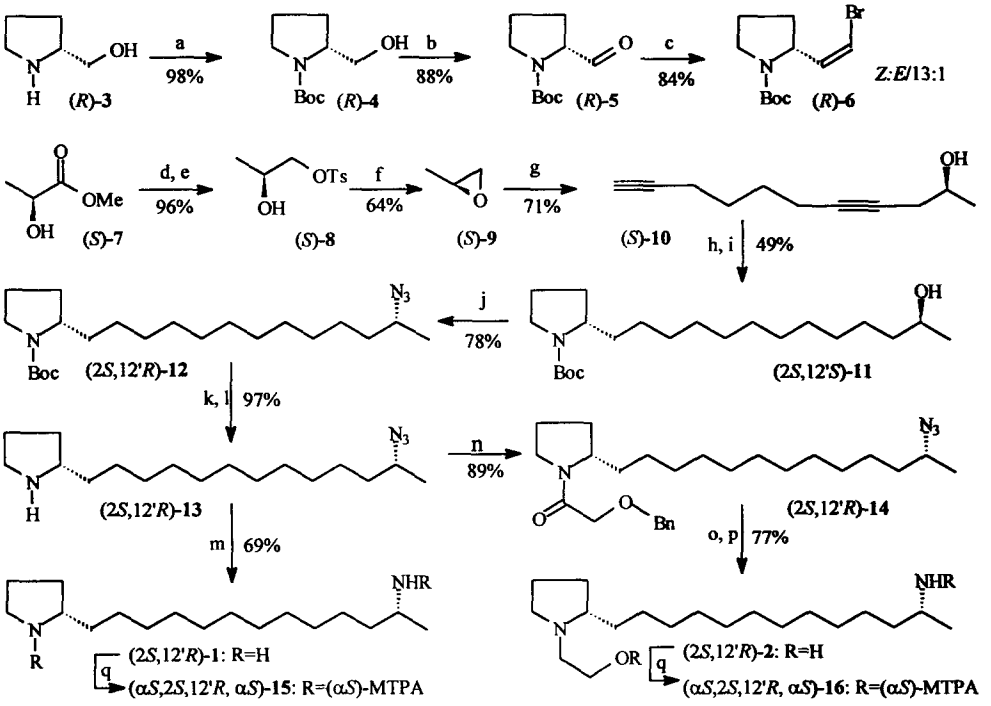
1



2

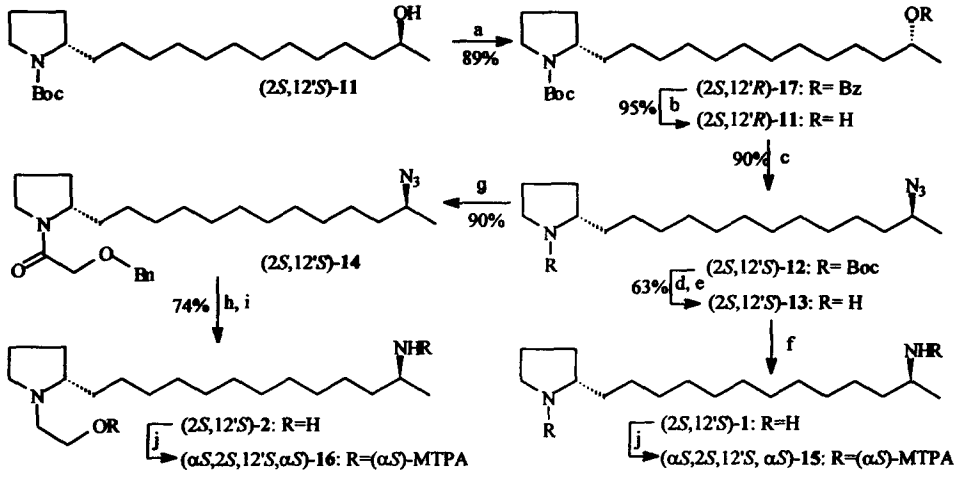
Our synthetic strategy for (2*S*,12′*R*)-**1** and (2*S*,12′*R*)-**2** featured a coupling of two chiral moieties to a linear α,ω-diyne chain at two termini. As shown in **Scheme 1**, chiral vinylic bromide (*R*)-**6** was prepared from (*R*)-2-pyrrolidinol (**3**) in three steps: protection of the amino group with Boc_2O,[6] oxidation with $Py.SO_3$,[7] and a Wittig coupling.[8] To obtain the other chiral moiety, (*S*)-methyl lactate (**7**) was reduced with $LiAlH_4$ and the primary alcohol group of the product was tosylated to give **8**.[9] Treatment of **8** with KOH afforded the volatile

epoxide **9** in high optical purity.[10] A selective opening of the epoxide ring with 1,7-octadiynyl lithium gave the desired diynol **10** in 71% yield. A palladium-catalyzed coupling of the vinyl bromide **(6)** to **(*S*)-10** provided the backbone of the target alkaloids.[11] This rather unstable intermediate, without isolation, was hydrogenated immediately over Pd/C, to give the saturated pyrrolidinol **(2*S*,12′*S*)-11** in 49 % overall yield. The conversion of the 12′*S*-hydroxyl group of **(2*S*,12′*S*)-11** into an azide to give **(2*S*,12′*R*)-12** was accomplished with N_3^-/DEAD.[12] Removal of the Boc group with HCl/EtOAc provided the pyrrolidine **(2*S*,12′*R*)-13** quantitatively,[13] which was subsequently reduced with $LiAlH_4$ to the desired alkaloid **(2*S*,12′*R*)-1** in 69% yield. For the synthesis of **(2*S*,12′*R*)-2**, **(2*S*,12′*R*)-13** was acylated with $BnOCH_2COCl$ to give the amide **(2*S*,12′*R*)-14** in 89% yield. The treatment of **(2*S*,12′*R*)-14** with $LiAlH_4$ not only reduced the azide moiety to the corresponding amine and the carbonyl group to a methylene group, but also unexpectedly removed the protecting group to some extent, providing the final product **(2*S*,12′*R*)-2** along with the undeprotected intermediate. Finally, this mixture was subjected to hydrogenolysis over Pd/C to yield **(2*S*,12′*R*)-2** in 77% overall yield.



Scheme 1. (a) $(t\text{-BuOCO})_2O$, CH_2Cl_2, 0 °C, 0.5 h, then 25 °C, 12 h; (b) $Py.SO_3$, DMSO, TEA, -10 to 15 °C, 15 min; (c) $Ph_3PCH_2Br_2$/NaN(TMS)$_2$; THF/$PhCH_3$/HMPA, -78 °C to 25 °C, 1 h; (d) $LiAlH_4$, THF, 25 °C, 1 h; (e) TsCl, TEA, CH_2Cl_2, -15 °C, 20 h; (f) KOH, H_2O, 3 h; (g) *n*-BuLi/1,7-octadiyne, Li_2CuCl_4, THF/HMPA, -20 °C, 12 h; (h) **6**, $Pd(Ph)_4$, CuI, *n*-$BuNH_2$, C_6H_6, 25 °C 12 h; (i) H_2/Pd 10% on carbon, THF, 48 h; (j) DEAD, $P(Ph)_3$, $(Ph)_2PON_3$, THF, 25 °C, 24 h; (k) HCl, EtOAc, 25 °C, 0.5 h; (l) KOH/H_2O, ether, 25 °C; (m) $LiAlH_4$, THF, 25 °C, 1 h; (n) $BnOCH_2COCl$, TEA, CH_2Cl_2, 0°C, 12 h; (o) $LiAlH_4$, THF, 50 °C, 12 h; (p) H_2/Pd 10% on carbon, 1 h; (q) (*R*)-Mosher chloride, pyridine, CH_2Cl_2, -20°C, 12 h.

The corresponding diastereomers, (2*S*,12′*S*)-**1** and (2*S*,12′*S*)-**2**, were prepared as outlined in **Scheme 2**. The inverted product (2*S*,12′*R*)-**11** was obtained from (2*S*,12′*S*)-**11** by a Mitsunobu reaction with DEAD/$C_6H_5CO_2H$ followed by alkaline hydrolysis.[14,15] The subsequent synthetic procedures were similar to those described for (2*S*,12′*R*)-**1** and (2*S*,12′*R*)-**2**.



Scheme 2. (a) DEAD, Ph_3P, $C_6H_5CO_2H$, THF, -50 °C, 0.5 h, then 25 °C, 2h; (b) KOH, MeOH, 25 °C, 12 h; (c) DEAD, Ph_3P, $(Ph)_2PON_3$, THF, 25 °C, 24 h; (d) HCl, EtOAc, 25 °C, 0.5 h; (e) KOH/H_2O, ether, 25 °C; (f) $LiAlH_4$, THF, 25 °C, 1 h; (g) $BnOCH_2COCl$, TEA, CH_2Cl_2, 0°C, 12 h; (h) $LiAlH_4$, THF, 50 °C, 12 h; (i) H_2/Pd, 10% on carbon, 1 h; (j) (*R*)-Mosher chloride, pyridine, CH_2Cl_2, -20°C, 12 h.

As reported previously,[5] the four stereoisomers of **1** can be distinguished by ^{1}H NMR analysis after attaching an MTPA moiety onto both the primary and secondary amino groups of **1**. Accordingly, the (*S*)-MTPA diamide of the synthetic (2*S*,12′*R*)-**1** [namely (α*S*,2*S*,12′*R*,α*S*)-**15**], was prepared using (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*R*)-MTPA chloride] (**Scheme 1**). ^{1}H NMR (500 MHz) analysis of this derivative showed that the spectral data of (α*S*,2*S*,12′*R*,α*S*)-**15** are congruent with those of (*S*)-MTPA diamide derived from the natural alkaloid **1**. In contrast, ^{1}H NMR data of the (*S*)-MTPA derivative (α*S*,2*S*,12′*S*,α*S*)-**15** prepared from (2*S*,12′*S*)-**1** (**Scheme 2**) showed a signal at δ 1.12 for the C-13′ methyl doublet, clearly distinguishable from the corresponding peak at δ 1.18 in the spectrum of the (*S*)-MTPA derivative prepared from the natural alkaloid **1**. Since neither (2*R*,12′*S*)-**1** nor (2*R*,12′*R*)-**1** is identical with the natural isomer, the absolute configuration of the natural alkaloid **1** was unambiguously established to be (2*S*,12′*R*), confirming our previous assignment.[5] This assignment is also consistent with the observed specific rotations {synthetic (2*S*,12′*R*)-**1**: $[\alpha]^{22}_D$ + 9.8°, c 0.25, $CDCl_3$; natural alkaloid **1**: $[\alpha]^{22}_D$ + 9.3°, c 0.15, $CDCl_3$}.[5]

Since no apparent interaction was observed between the two chiral centers of alkaloid **1** or its MTPA derivatives[5], we hypothesized that there is also no significant mutual influence between the two chiral terminal moieties of alkaloid **2** or its MTPA derivatives as well. The (*S*)-MTPA derivatives of both the natural alkaloid **2** and synthetic (2*S*,12′*R*)-**2** were prepared using (*R*)-MTPA chloride (**Scheme 1**) and a ^{1}H NMR analysis clearly indicated that spectral data of (α*S*,2*S*,12′*R*,α*S*)-**16** are indistinguishable from those of the (*S*)-

MTPA derivative from the natural alkaloid **2** and the same derivatizing reagent [CH_3-C-12′: (δ 1.18, J = 6.8 Hz), O-CH_2-C-1′′: (δ 4.57, 1H, ddd, J = 11.3, 7.3, 5.5 Hz; δ 4.36, 1H m)]. On the other hand, the spectral data of (α*S*,2*S*,12′S,α*S*)-**16** [CH_3-C-12′: (δ 1.12, J = 6.8 Hz), O-CH_2-C-1′′: (δ 4.57, 4.36)] derived from the synthetic sample (2*S*,12′S)-**2** and (*R*)-MTPA chloride are different from those of the (*S*)-MTPA derivative from the natural alkaloid **2** (**Scheme 2**). Therefore, the absolute configuration of the natural alkaloid **2** at position C-12′ could be assigned to be (12′*R*), as anticipated.[5] To assign the absolute configuration of alkaloid **2** at position C-2, we prepared the (α*R*)-MTPA derivative, the enantiomer of (α*S*,2*R*,12′*R*,α*S*)-**16**, from synthetic (2*S*,12′*S*)-**2**, and (*S*)-MTPA chloride. The ^{1}H NMR spectrum of (α*R*,2*S*,12′S,α*R*)-**16** [CH_3-C-12′: (δ1.18, J = 6.8 Hz), O-CH_2-C-1′′: (δ 4.50, 1H, dt, J = 11.1, 6.7 Hz; δ 4.34, 1H, dt, J = 11.0, 6.7 Hz)], which must show identical ^{1}H NMR data to those of its enantiomer (α*S*,2*R*,12′*R*,α*S*)-**16**, does not match in the δ 4.30-4.60 (position C-2′′) to that of (α*S*)-MTPA derivative from the natural **2**. These results revealed that (α*S*,2*R*,12′*R*,α*S*)-**16** is in fact the C-2 epimer of the (α*S*)-MTPA derivative from the natural alkaloid **2**. In this way, the absolute stereochemistry of natural alkaloid **2** was shown to be (2*S*,12′*R*)-**2**. This conclusion is supported by specific rotation values {synthetic (2*S*,12′*R*)-**2**: $[\alpha]^{22}_D$ +37.5°, c 0.64, $CDCl_3$; natural alkaloid **2**: $[\alpha]^{22}_D$ +38.8°, c 0.18, $CDCl_3$}.[5]

In summary, the *Epilachna* alkaloids (2*S*,12′*R*)-**1** and (2*S*,12′*R*)-**2**, along with their non-natural diastereomers, (2*S*,12′*S*)-**1** and (2*S*,12′*S*)-**2**, were synthesized, and the absolute configurations of the naturally occurring **1** and **2** were unambiguously assigned as (2*S*,12′*R*).

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