A new phenylethyl alkyl amide from the *Ambrostoma quadriimpressum* Motschulsky

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**Abstract**

A new phenylethyl alkyl amide, (10R)-10-hydroxy-N-phenethyloctadecanamide (1), was isolated from the beetle *Ambrostoma quadriimpressum* Motschulsky. The structure of the amide was determined by NMR and MS. The absolute configuration of compound 1 was confirmed by an asymmetric total synthesis, which was started from L-glutamic acid. The construction of the aliphatic chain was accomplished by the selective protection of the hydroxy groups and two-time implementation of the Wittig olefination reaction.

**Introduction**

The leaf beetle *Ambrostoma quadriimpressum* Motschulsky (Coleoptera: Chrysomelidae) is a serious pest of elm that exists primarily in north-eastern China. Adult beetles hibernate over the winter and emerge in the spring when their host plant is most succulent. The developmental stages of beetles are able to defend themselves against predatory attacks, and this defence is associated with chemical defensive compounds.

Insects are able to produce a vast array of biologically active secondary metabolites, which are used for defence purposes or as pheromones. In particular, the chemical defensive phenomenon of the beetle has been observed in Coleoptera [1,2]. Over the last few years, many research efforts have concentrated on chemical communication among beetles [3-6]. Only a limited number of semiochemicals [7-13] are known in leaf beetles. To the best of our knowledge, fatty acid amides from terrestrial insects have not been reported so far. In addition, certain marine organisms [14,15] and mushrooms [16,17] are the only two natural sources of phenylethyl alkyl amides with remarkable activities, such as cytotoxicity to murine leukemia cells (P-388) [14] and protective activity against endoplasmic reticulum stress-dependent cell death [17]. In the current study, we report the isolation of (10R)-10-hydroxy-N-phenethyloctadecanamide (1), a possible chemical defensive compound from the beetle *A. quadriimpressum*, and confirmation of its absolute configuration by total synthesis.
Results and Discussion

Isolation of (10R)-10-hydroxy-N-phenethyl-octadecanamide (1)

Petroleum ether extracts from adult *A. quadriimpressum* were purified repeatedly by means of silica gel column chromatography. Compound 1 was obtained as a white solid. An ion peak at *m/z* 403 and an [M – H2O]+ peak at *m/z* 385 in the ElMS suggested the presence of an OH group. The molecular formula of 1 was confirmed as C26H45NO2 (404.3529, [M + H]+) by HRMS–ESI analysis. The 1H NMR spectrum showed aromatic protons [δ 7.33–7.18 (m, 5H)], CH2 groups [δ 3.52 (q, *J* = 6.4 Hz, 2H), 2.82 (t, *J* = 6.8 Hz, 2H), 2.11 (t, *J* = 7.2 Hz, 2H), 1.58–1.05 (m, 24H)], an amide proton [δ 5.39 (br s, 1H)], a CH group [δ 3.58 (br m, 1H)] and an Me group [δ 0.88 (t, *J* = 6.8 Hz, 3H)]. The COSY spectrum showed that compound 1 had a coupled proton spin system CH2CH2NH (δ 5.39, 3.52, 2.82 ppm). Correlations obtained from an HMBC spectrum provided partial evidence to assign a phenethylamine moiety. The location of the OH group in the aliphatic chain was assigned by EIMS analysis; specifically, evidence was provided by a strong [M – 113]+ peak at *m/z* 290 corresponding to the loss of C8H17 (Scheme 1). Hence, compound 1 was confirmed as 10-hydroxy-N-phenethyl-octadecanamide. In addition, compound 1 showed positive optical rotation ([α]20D +38.5 (c 0.13, CHCl3)). To confirm the absolute configuration at C(10), an asymmetric total synthesis was performed.

Synthesis of (10R)-10-hydroxy-N-phenethyl-octadecanamide (1)

The retrosynthetic analysis of compound 1 is outlined in Scheme 2. The target molecule is disconnected into two fragments, 2 and 3. The intermediate fragment 2 would be readily...
prepared from the commercially available ε-caprolactone. The crucial fragment 3 would be constructed from the intermediate 4 through Wittig olefination. The polyhydroxy compound 4 would be obtained from L-glutamic acid by selective protection.

Our synthesis started from the ready available L-glutamic acid, as shown in Scheme 3. On the basis of the preceding literature [18-20], the hydroxy diester 5 was obtained in 35% yield (BnOH, H₂SO₄; NaNO₂, AcOH; CH₂N₂). Protection of the secondary alcohol with the TBDPS group gave compound 6 in quantitative yield. The following debenzylation and reduction with BH₃·THF afforded alcohol 7 in 95% isolated yield. Then primary alcohol 7 was protected again with TBDMSCl to give compound 8 in 100% yield. An initial attempt to synthesize compound 9 by reduction of 8 with LiAlH₄, however, resulted in the deprotection of the TBDPS group. Alternatively, the alcohol 9 could be obtained by reduction with diisobutylaluminium hydride (Dibal-H) in 86% yield, which was then converted to the corresponding aldehyde 10 by oxidation with PCC. Wittig olefination of aldehyde 10 with n-heptyldienetriphenylphosphonium bromide in the presence of n-BuLi gave adduct 11 as an (E/Z)-mixture (E/Z = 1:5) in 75% yield. Selective removal of the TBS group with pyridinium tosylate gave 12 in 98% yield [21].

With the alcohol 12 in hand, our next step was to synthesize the phosphonium salt 15 [22,23] (Scheme 4). Therefore, ε-caprolactone was hydrolyzed with 5% NaOH to give hydroxy acid 13 in quantitative yield, which was reacted with PBr₃ in CH₂Cl₂ to afford compound 14 in 82% yield. Treatment of 14 with PPh₃ in CH₃CN gave the phosphonium salt 15 in 98% yield.

The construction of the aliphatic chain was achieved according to the synthetic plan shown in Scheme 5. Oxidation of the

**Scheme 3:**

(a) BnOH, H₂SO₄; NaNO₂, AcOH; CH₂N₂, 35% in 3 steps. (b) TBDPSCI, imidazole, 100%. (c) Pd/C, H₂; BH₃·THF, 95% in 2 steps. (d) TBDMSCl, imidazole, 100%. (e) Dibal-H, 86%. (f) PCC, 94%. (g) C₇H₁₅PPh₃Br, n-BuLi, 75%. (h) PPTS, 98%.

**Scheme 4:**

(a) 5% NaOH, 100%. (b) PBr₃, 82%. (c) PPh₃, 98%.

**Scheme 5:**

(a) PCC, 96%. (b) LDA, 15, 72%. (c) DCC, DMAP, phenylethylamine, 80%. (d) Pd/C, H₂, 100%. (e) TBAF, 93%.
alcohol 12 with PCC afforded the corresponding aldehyde 16, followed by a Wittig reaction with salt 15 in the presence of two equiv LDA, which afforded the adduct 17 as an (E/Z)-mixture in 72% yield. The E/Z isomers of 17 were not separated because the olefin geometry has no effect on the synthesis. Therefore, compound 17 was directly converted to the amide 18 with DCC and DMAP in 80% yield. Reduction of the C=C double bond by hydrogenation with Pd/C gave compound 19 in quantitative yield, which was transferred to the final product (R)-1 in 93% yield by removal of the TBDPS group. Both NMR and EIMS data were in complete agreement with those of the natural product 1. In addition, the synthetic compound (R)-1 showed positive optical rotation ([α]D+37.2 (c 0.85, CHCl3)), suggesting that the absolute configuration of natural product 1 is to be assigned (R).

Conclusion
In summary, a new natural product, (10R)-10-hydroxy-N-phenethyloctadecanamide (1) from Ambrostoma quadriimpressum Motschulsky was identified and synthesized. Further studies on the biological roles of fatty acid amides are currently being performed by our group.

Experimental
General Methods: Commercial, spectral-grade solvents were used for the experiments unless otherwise stated. Infrared spectra were recorded on a Perkin–Elmer 1710 Fourier transform spectrometer. Low-resolution mass spectra were obtained from Agilent 7890A-5975C GC–MS by means of electron impact (EI) ionization at 70 eV. High-resolution mass spectra were recorded on an Agilent 1200-6520 Q-TOF electrospray mass spectrometer. Experimental procedures for the synthesis of compound 1.

Supporting Information
Supporting Information File 1
Detailed experimental procedures for the synthesis of compound 1.

Acknowledgements
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Supporting Information
Supporting Information File 1
Detailed experimental procedures for the synthesis of compound 1.
[http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-7-158-S1.pdf]
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