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Note

# Biosynthesis of Quinoline by a Stick Insect

Athula B. Attygalle,* Kithsiri B. Hearth, Vikram K. Iyengar, and Randy C. Morgan

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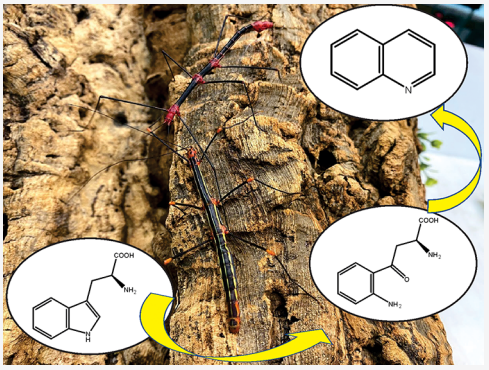
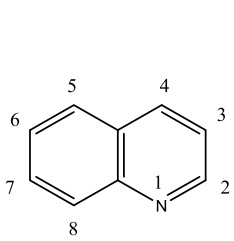
**ABSTRACT:** The Peruvian stick insect *Oreophoetes peruana* is the only known animal source for unsubstituted quinoline in nature. When disturbed, these insects discharge a defensive secretion containing quinoline. Analysis of samples obtained from L-[2′,4′,5′,6′,7′-$^{2}H_5$]tryptophan-fed stick insects demonstrated that the insects convert it to [5,6,7,8-$^{2}H_4$]quinoline by removing the 2′-CH moiety in the indole ring of tryptophan. Analogous experiments using L-[1′-$^{15}$N]tryptophan and L-[1′-$^{15}$N,$^{15}NH_2$]tryptophan showed that the indole-N atom is retained while the α-amino group is eliminated during the biosynthesis. Mass spectra recorded from quinoline derived from [2-$^{13}C_1$]tryptophan-fed insects indicated that the α-carbon atom of tryptophan is incorporated as the C-2 atom of the quinoline ring.

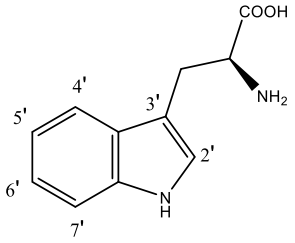




The so-called stick insects of the order Phasmatodea are typically cryptic, armed with spines, or protected by camouflage by resemblance to twigs or leaves. Because they are mostly wingless and slow moving, these animals are expected to be vulnerable to predators. In this respect, the Peruvian fire stick, *Oreophoetes peruana* (Floyd, 1993),[1] is atypical because these insects are strikingly colored. The males of *O. peruana* are black with conspicuous bright red patches, while females have orange markings with longitudinal yellow stripes. Presumably, *O. peruana*, can exhibit its aposematic coloration blatantly because it is protected by the allomones present in their defensive glands. Previously, we reported that *O. peruana* when disturbed ejects a white malodorous fluid from its defensive glands in the prothorax, and quinoline (**1**) was identified as the major constituent in the secretion.[2]



**(1)**



**(2)**

Quinoline (**1**) is an aromatic compound first identified from coal tar by Runge in 1834.[3] About a century later, Späth and Pikl[4] reported it as one of the trace constituents (about 0.003%) of angostura bark (*Cusparia trifoliata*). Many compounds that have a quinoline-core structure such as quinine[5] and chloroquine[6] are used as active ingredients in pharmaceutical preparations.[7,8] Although many quinoline-based alkaloids are known from plants, animals, and microbial sources,[9−16] unsubstituted quinoline as an animal natural product has been identified only from *O. peruana*.[2]

A compound that bears the quinoline core, 6-hydroxykynurenic acid, has been shown to be biosynthesized from L-tryptophan (**2**) in *Nicotiana tabacum*.[16] In an analogous manner, 8-hydroxyquinoline-2-carboxylic acid found in the foregut content of the cotton leafworm *Spodoptera littoralis* has been demonstrated to be generated from tryptophan via kynurenine, kynurenic acid, and quinaldic acid.[17] Xanthurenic acid (4,8-dihydroxyquinoline-2-carboxylic acid) found in *Drosophila melanogaster*[10,11] and several mosquito species[13] has been shown to originate from L-tryptophan via 3-hydroxykynurenine.[18−21] As many quinolinic compounds have been shown to be products of tryptophan metabolism,[22,23] we hypothesized that unsubstituted quinoline in *O. peruana* could be biosynthesized by an oxidative transformation of tryptophan. Herein, we report on the evaluation of biosynthetic pathways for the formation of quinoline from tryptophan, using several stable isotope-labeled precursors such as L-[2′,4′,5′,6′,7′-$^{2}H_5$]tryptophan, L-[1′-$^{15}$N]tryptophan, L-[1′-$^{15}$N,$^{15}NH_2$]tryptophan, and [2-$^{13}C_1$]tryptophan.

The analysis of an extract made from the defensive secretion of *O. peruana* by GC-MS showed essentially one chromato-

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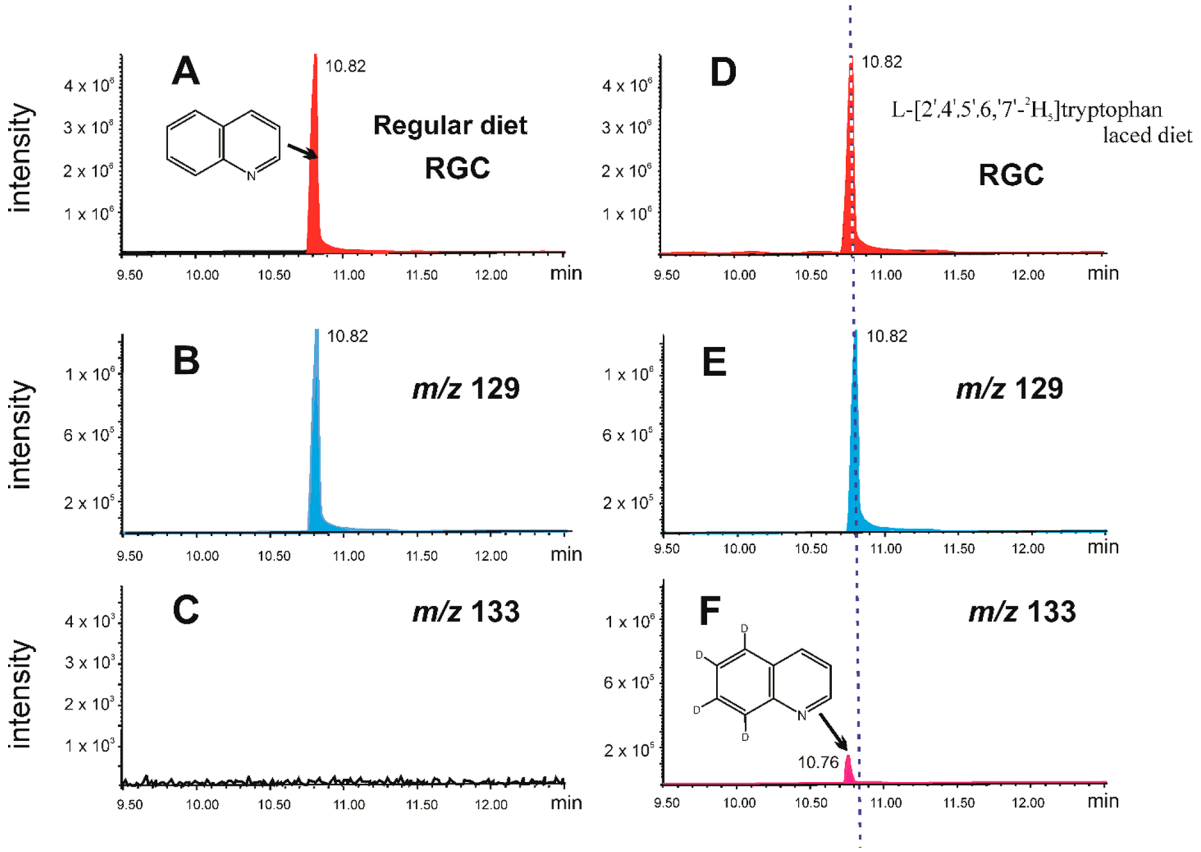


**Figure 1.** Reconstructed gas chromatogram (RGC) and ion-intensity profiles extracted from acquired data for the *m*/*z* 129 (B) and 133 (C) from a defensive secretion extract prepared from *Oreophoetes peruana* maintained on a regular diet. Panels D (RGC), E (*m*/*z* 129), and F (*m*/*z* 133) show analogous profiles recorded from a defensive secretion extract prepared from insects maintained on a diet of fern leaves laced with L-[2′,4′,5′,6,′7′-$^{2}H_5$]tryptophan. Analytical conditions are described in the text.

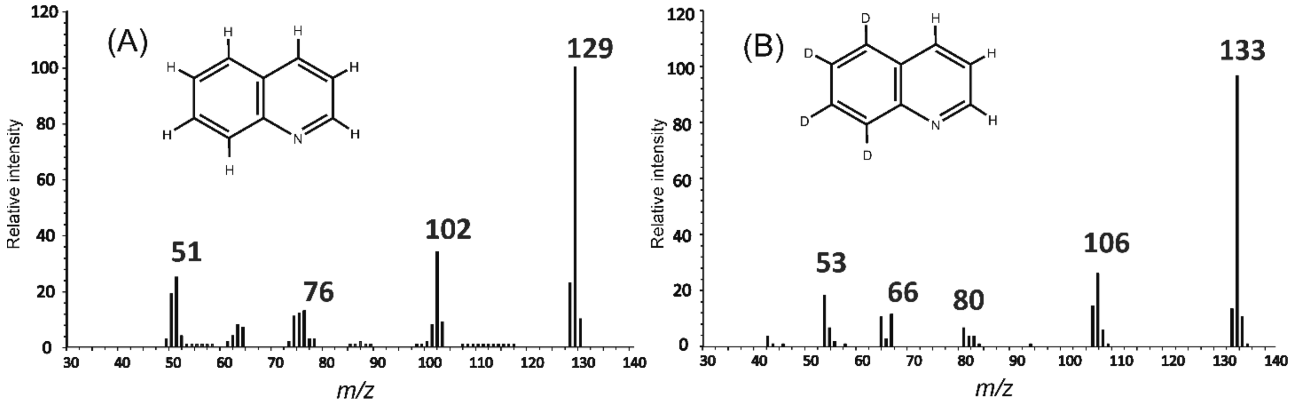


**Figure 2.** Electron-ionization (70 eV) mass spectra corresponding to the quinoline peak in Figure 1A (A) and that for the deuteriated compound that eluted immediately before the quinoline peak (B).

graphic peak, which represented quinoline (**1**) as reported previously (Figure 1A).[2] Data acquired from extracts made from the defensive fluid of insects fed with L-[2′,4′,5′,6,′7′-$^{2}H_5$]tryptophan showed a similar chromatographic profile (Figure 1D). However, a careful scrutiny of the mass spectra recorded from the sample obtained from L-[2′,4′,5′,6,′7′-$^{2}H_5$]tryptophan-fed insects showed the presence of an extra mass peak at *m*/*z* 133, in addition to that at *m*/*z* 129 expected for the molecular ion of quinoline. Time–intensity profiles for *m*/*z* 129 and 133 ions, extracted from the acquired GC-MS data, confirmed the presence of an additional chromatographic peak for a compound that eluted slightly before the quinoline peak (Figure 1F). In fact, it is well known that deuteriated isotopologues elute earlier than their non-labeled counterparts under GC conditions.[24−26] Evidently, the earlier eluting peak we observed represented a deuterio-isotopologue of quinoline. By following a careful background subtraction protocol, we were able to obtain a representative mass spectrum for the deuteriated compound (Figure 2). Because the nominal mass of the new compound was 133 u (Figure 2B), which is four mass units higher than that of quinoline (Figure 2A), it was evident that of the five deuterium


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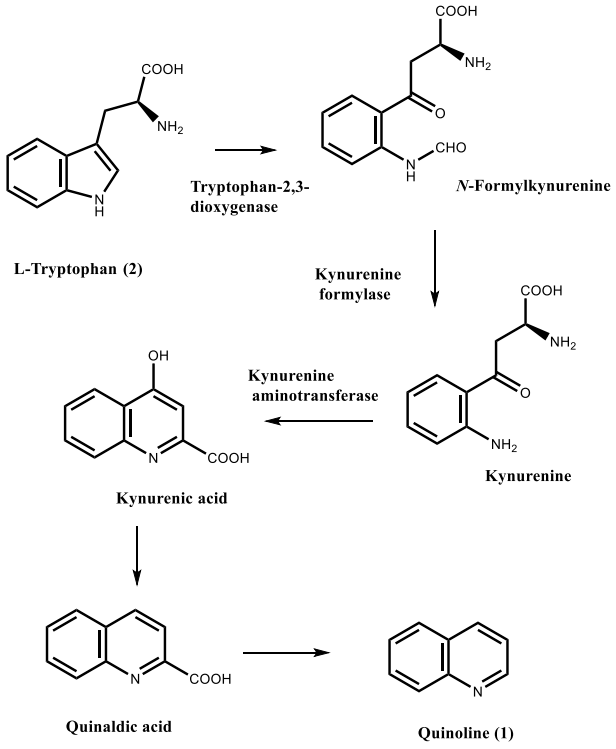
atoms initially present in the L-[2′,4′,5′,6,7′-$^2H_5$]tryptophan, only four of them have been incorporated into the labeled product (Figure 2B). Moreover, in the spectrum in Figure 2B, a fragment-ion peak was observed at $m/z$ 106 for an expulsion of a 27 Da hydrogen cyanide molecule from the precursor $m/z$ 133 ion. Based on rationalizations documented for the mass spectral fragmentation of quinoline under electron-ionization conditions, the hydrogen atom for the lost HCN originates from the heterocyclic ring of quinoline.[27] Thus, it was apparent that all deuterium atoms on the phenylene ring of L-[2′,4′,5′,6,7′-$^2H_5$]tryptophan are retained in the course of its conversion to labeled quinoline, while the 2′-$^2$H atom is eliminated (Supporting Information Scheme S1).

The electrospray-ionization mass spectrum recorded from the defensive secretion of *O. peruana* maintained on a diet laced with L-[2′,4′,5′,6,7′-$^2H_5$]tryptophan provided further support to confirm the proposed biosynthetic pathway. The positive-ion mass spectrum acquired from a sample prepared in acidified MeCN/$H_2O$ extract showed a mass peak at $m/z$ 134 for protonated quinoline-$d_4$ (Figure S1 and Scheme S1). Moreover, gas chromatograms recorded from extracts made from the secretion obtained from insects fed with L-[1′-$^{15}$N]tryptophan and L-[1′-$^{15}$N,$^{15}NH_2$]tryptophan also showed a chromatographic peak at a retention time similar to that obtained from insects on a regular diet (Figure 1A). However, it was not straightforward to monitor the formation of a labeled quinoline from $^{15}N_1$-labled tryptophan because the expected product [$^{15}N_1$]quinoline is isobaric to natural [$^{13}C_1$]quinoline. Nevertheless, definitive deductions could be ascertained by extracting ion intensity data from the acquired GC-MS file. The mass spectrum recorded from native quinoline showed two mass peaks at $m/z$ 129 and 130 for $M^{+\bullet}$ and $[M + 1]^{+\bullet}$ ions, respectively (Figure 2A). The area ratio of peaks in mass chromatograms extracted for $m/z$ 129 and 130 ions for native quinoline is about 100:10 (Figure S2). In contrast, the mass spectrum corresponding to the composite quinoline peak and the extracted ion chromatograms recorded from the defensive secretion obtained after feeding L-[1′-$^{15}$N]tryptophan manifested an $m/z$ 129 to 130 peak intensity ratio of about 100:45 (Figures S3 and S4). Analogous results were obtained from L-[1′-$^{15}$N,$^{15}NH_2$]tryptophan-fed insects as well (Figures S5 and S6). The relative increase of the intensity of the $m/z$ 130 peak confirmed that the indole nitrogen atom is retained when tryptophan is transformed to quinoline.

Further biosynthetic experiments were conducted with [2-$^{13}C_1$]tryptophan. The mass spectrum corresponding to the quinoline peak and the extracted ion chromatograms recorded from the defensive secretion obtained after feeding [2-$^{13}C_1$]-tryptophan manifested an $m/z$ 129 to 130 peak intensity ratio of about 100:75, which compared to a 100:10 ratio for native quinoline, indicating a very high degree of incorporation of the experimentally provided stable-isotope label (Figures S7 and S8). Although the mass spectrum recorded was a composite of [2-$^{13}C_1$]quinoline and all other isotopomers of natural [$^{13}C_1$]quinoline, the $m/z$ 129 to 130 peak intensity ratio of 100:75 confirmed that the [$^{13}C_1$]-isotopologue that contributed primarily to the spectrum was the [2-$^{13}C_1$]-isotopologue of quinoline. Moreover, the intense peak at $m/z$ 102 (Figure S7) for H$^{12}$CN or H$^{13}$CN losses from $m/z$ 129 and 130 ions, respectively, indicated that the incorporated $^{13}$C-label was primarily located at the C-2 position of the quinoline ring.[27]

By taking similar pathways proposed for the biosynthesis of quinoline derivatives in other systems and our experimental data into consideration, we propose that quinoline in *O. peruana* is made from tryptophan as outlined in Scheme 1.

**Scheme 1. Conversion of L-Tryptophan (2) to Quinoline (1)**




Although the putative intermediates were not isolated, we presume that the 2,3-double bond of the pyrrole ring of tryptophan is cleaved by a tryptophan 2,3-dioxygenase to form *N*-formylkynurenine.[28] A pathway to remove the formyl group by a kynurenine formylase to form kynurenine has been reported by Paglino et al.[28] A kynurenine aminotransferase can then cyclize kynurenine to kynurenic acid (Scheme 1). Alternatively, the ketone moiety in kynurenine can be reduced before cyclization. In an earlier study, Slaytor et al. has demonstrated that in *Nicotiana tabacum* tryptophan is converted to 8-hydroxykynurenic acid.[16] Eventually, a deoxygenation of kynurenic acid can yield quinaldic acid. Previously, rabbits have been shown to be able to deoxygenate kynurenic acid or xanthurenic acid to quinaldic acid and 8-hydroxyquinaldic acid, respectively.[29] Apparently, *O. peruana* has evolved to decarboxylate quinaldic acid to form quinoline as the final product.

More than 3000 species of stick insects exist, and the defensive chemistry of some of them has been investigated.[2,30−33] However, this is the first report on the biosynthetic origin of defensive allomones of any stick insect. Although the intermediates participating in the conversion process were not isolated, our data unambiguously demonstrate that the stick insect *O. peruana* is able to convert tryptophan to quinoline.

## EXPERIMENTAL SECTION

**Insect Samples.** *Oreophetes peruana* specimens used in this study originated from a colony maintained at the Cincinnati Zoo &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Botanical Garden, OH, USA. They were caged in individual plastic containers (10 cm × 10 cm × 10 cm) and fed routinely on a diet of *Nephrolepis exaltata* (Boston fern). The defensive secretion of *O. peruana* was "milked" by holding specimens by hand and collecting the fluid that oozed from the glands into glass capillary tubes.

**Chemicals.** L-[2′,4′,5′,6′,7′-$^{2}H_5$]Tryptophan, L-[1′-$^{15}N$]-tryptophan, L-[1′-$^{15}N$,$^{15}NH_2$]tryptophan, and [2-$^{13}C_1$] glycine were purchased from Cambridge Isotope Laboratories, Inc. Indole-3-aldehyde was obtained from Sigma-Aldrich Chemical Co. DL-[2-$^{13}C_1$]Tryptophan was synthesized by adapting the procedures described by Ellinger and Flamand[34] (see Supporting Information). Stock solutions of each test chemical were made in deionized $H_2O$ (1 mg/100 μL).

For feeding experiments, each putative precursor chemical was applied as aqueous solutions (about 1 mg/0.1 mL) on Boston fern leaves, the solvent was allowed to evaporate, and the leaves were offered to *O. peruana*.

**Chemical Analysis.** After 5−7 days of feeding, defensive secretion samples were collected, extracted with $CH_2Cl_2$, and analyzed by gas chromatography mass spectrometry on an HP 5890 GC coupled to a 5970 MSD mass analyzer operated at 70 eV, using a 30 m × 0.22 mm fused-silica column coated with DB-5 (J & W Scientific). For routine analysis, the GC oven temperature was kept at 60 °C for 4 min, raised at 8 °C $min^{-1}$ to 250 °C, and held at this temperature for 5 min.

Analogously, extracts prepared in $CH_3CN$ and $H_2O$ (50:50 v/v, with a drop of 0.1% formic acid) were analyzed on a Quattro 1 instrument (Micromass) by electrospray-ionization mass spectrometry at a sampling-cone voltage setting of 20 V.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00945.

Experimental data on the synthesis of labeled compounds, additional mass spectra and chromatograms, and proposed biosynthetic schemes (PDF)

## ■ AUTHOR INFORMATION


### Corresponding Author

**Athula B. Attygalle** − *Department of Chemistry and Chemical Biology, Stevens Institute of Technology, Hoboken, New Jersey 07030, United States*; orcid.org/0000-0002-5847-2794; Phone: 201-216-5575; Email: athula.attygalle@stevens.edu

### Authors

**Kithsiri B. Hearth** − *Department of Chemistry and Chemical Biology, Stevens Institute of Technology, Hoboken, New Jersey 07030, United States*

**Vikram K. Iyengar** − *Department of Biology, Villanova University, Villanova, Pennsylvania 19085, United States*

**Randy C. Morgan** − *Insectarium, Cincinnati Zoo & Botanical Garden, Cincinnati, Ohio 45220, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jnatprod.0c00945


### Notes

The authors declare no competing financial interest.

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