N-Methylquinolinium 2-carboxylate, a Defensive Betaine from *Photuris versicolor* Fireflies[1]

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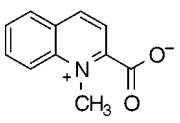
From whole body extracts of *Photuris versicolor* fireflies, the defensive betaine *N*-methylquinolinium 2-carboxylate (**1**) was isolated and characterized on the basis of spectroscopic data and comparison with a synthetic sample.

Fireflies (Coleoptera: Lampyridae) are known to be distasteful to a number of predators.[2−5] In fireflies of the genus *Photinus* (*P. ignitus*, *P. pyralis*, *P. marginellus*), this distastefulness has been shown to be attributable, in part at least, to steroidal pyrones (lucibufagins) present systemically in the beetles.[5−8] Fireflies of another genus, *Photuris*, are also protected by lucibufagins, but they acquire the compounds largely by feeding on *Photinus*. Specifically, it is the female *Photuris* that acquires lucibufagins, by luring and capturing the male *Photinus*.[9] We found females of one species of *Photuris*, *P. versicolor*, to contain another defensive substance, distinct from lucibufagins, present in their blood, whether they fed on *Photinus* or not. This compound, the betaine *N*-methylquinolinium 2-carboxylate (**1**), not known as a natural product, is present in male and larval *P. versicolor* as well and appears to be produced endogenously by this species. Data that we will be presenting elsewhere demonstrate that female *P. versicolor* transmit a fraction of their betaine, together with some of their acquired lucibufagin, to the eggs, providing these with protection as a result. The betaine constitutes an important addition to this defensive endowment (unpublished data). We here report on the isolation and characterization of this betaine.

Whole body extracts of female *P. versicolor* fireflies were analyzed by reversed-phase HPLC and by coupled HPLC/MS (Figure 1). In the initial HPLC analyses, several components of moderate polarity were identified as lucibufagins.[5−8] This finding was not unexpected, inasmuch as the female *Photuris* collected in the field must have had the opportunity to sequester lucibufagins from their *Photinus* male prey.[9] In the hope of finding additional, nonsteroidal defensive compounds, we turned our attention to a major, more polar, UV-active component in this extract. This polar compound was isolated and purified by reversed-phase HPLC, and identified as next described.



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When examined by positive-ion electrospray mass spectrometry, the purified material showed ions at *m*/*z* 188, 144, and 128, in addition to an ion at *m*/*z* 210. These data

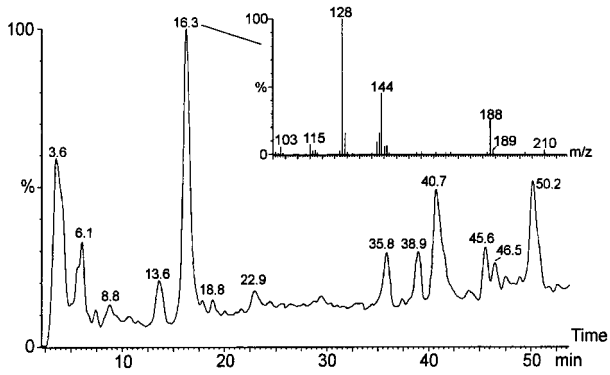

Figure 1. LC/MS trace (TIC) of a crude extract of *P. versicolor* fireflies. *N*-methylquinolinium *2*-carboxylate (**1**) elutes at 16.27 min (electrospray MS data shown). Components at 13.61 and 22.95 min are phenylalanine and tryptophan, respectively. Earlier eluting compounds are a mixture of several amino acids. Less polar compounds eluting at 35.80 min and later are sequestered lucibufagins.[9]

are easily rationalized by assuming that the *m*/*z* 188 ion represents the protonated molecular ion $[M + 1]^+$ and that the *m*/*z* 210 ion corresponds to a sodium ion adduct $[M + Na]^+$. HRFABMS showed the *m*/*z* 188 ion to have the molecular formula $C_{11}H_{10}NO_2$ (calcd 188.0712, found 188.0712), yielding the molecular formula $C_{11}H_9NO_2$ for the unprotonated unknown.

The UV spectrum of this compound, obtained during HPLC analysis by a diode-array detector, showed maxima at $\lambda = 240$ and 323 nm, indicating the presence of either a polycyclic aromatic system or some other extended conjugated chromophore.

A definite identification was made on the basis of ^1H and ^{13}C NMR spectroscopic studies. In its ^1H NMR spectrum, the unknown showed the presence of six aromatic protons and a methyl group attached to a heteroatom (Table 1). DQF−COSY spectra showed that the six aromatic protons belonged to two separate spin systems of four and two protons. ^{13}C NMR spectroscopy indicated the presence of 11 carbon atoms (Table 1), nine of them in the aromatic region (δ 118.4−155.5), one falling in the carboxyl region (δ 166.6), and one sp^3 carbon attached to a heteroatom (δ 41.2). The seven proton-bearing carbons were related to their protons by a gHMQC experiment. Of the four quaternary carbon atoms, two (δ 128.6 and 138.3) corresponded to bridgehead carbons of a bicyclic system, as indicated by their gHMBC correlations with protons from both aromatic spin systems (Table 1; Figure 2). The protons of the methyl group at δ 4.54 showed two gHMBC correlations, corresponding to the bridgehead carbon C-8a and the quaternary carbon C-2. In addition, C-2 showed gHMBC

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Table 1. ^{1}H (500 MHz) and ^{13}C (100 MHz) Data of *N*-Methylquinolinium 2-carboxylate (**1**)

		^{1}H data				
position	^{13}C data δ (ppm)	δ (ppm)	mult	int	*J* (Hz)	gHMBC correlations (C-position)
2	155.5					
3	119.4	7.91	d	1H	8.5	C-2, 4a, 10
4	148.0	9.07	d	1H	8.5	C-2, 5, 8a
4a	128.6					
5	130.4	8.31	dd	1H	8.1, 1.3	C-4, 7, 8a
6	129.7	7.99	ddd	1H	8.1, 7.1, 0.6	C-4a, 8
7	136.7	8.24	ddd	1H	9.0, 7.1, 1.3	C-8a
8	118.4	8.40	dd	1H	9.0, 0.6	C-4a, 6, 7
8a	138.3					
9	41.2	4.54	s	3H		C-2, 8a
10	166.6					

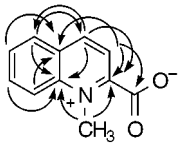

Figure 2. Relevant long-range H−C correlations observed in the gHMBC spectrum of **1**.

correlations with the protons at C-3 and C-4. Hence, the methyl group is attached to the nitrogen in a six-membered heterocycle, establishing the *N*-methylquinolinium system. The last carbon to be assigned (δ 166.6) showed a gHMBC correlation with the proton at C-3, indicating its position as a substituent of C-2. Its chemical shift corresponded to a carboxylic carbon atom, and only a carboxylate moiety satisfies the molecular formula as determined by HR-FABMS. These data unambiguously identify the major polar component of the *Photuris* extract as *N*-methylquinolinium 2-carboxylate (**1**).

To confirm this conclusion, a sample of **1** was prepared as described by Quast et al.[10] The synthetic material and the natural product showed indistinguishable NMR and mass spectra, as well as identical HPLC retention times. Examination (HPLC retention time and UV spectra) of extracts of male and larval *P. versicolor* established the presence of **1** in these life stages as well.

Although the quinoline nucleus occurs in a variety of plant alkaloids, quinoline-based secondary metabolites do not appear commonly in the animal kingdom.[11] Nonetheless, quinoline itself was reported very recently as the chief constituent of the defensive secretion of a phasmid insect,[12] and quinoline derivatives had been isolated from certain dytiscid, lycid, and coccinellid beetles.[13−15] It seems clear that this heterocyclic nucleus falls within the insects' biosynthetic repertoire.

Experimental Section

General Experimental Procedures. Preparative HPLC was performed using a semipreparative column (Supelco LC-18-DB, 25 cm × 10 mm i.d., 5 μm) (Supelco, Bellefonte, PA), a dual pump system (Waters M-45 and 510 models), an L4000 Hitachi UV detector (λ = 300 nm), and a HP 3396A integrator. The column was eluted initially with 10% CH_3CN in H_2O, increasing the amount of CH_3CN to 25% over a period of 55 min. The flow was 3.2 mL/min, and the runs were monitored at λ = 300 nm.

^{1}H, gHMQC, gHMBC, and DQF−COSY NMR spectra were recorded on a Varian Unity 500 spectrometer (500 MHz for proton; 126 MHz for ^{13}C). ^{13}C NMR spectra were obtained using a Varian XL 400 instrument (100 MHz). Spectra were obtained in D_2O. Residual HDO was used as ^{1}H reference (4.80 ppm), and 1 μL CH_3CN was added to the sample for ^{13}C reference (118.7 ppm).

LC/MS data were obtained with a HP 1090 HPLC instrument linked to a diode-array detector (HP) and a Quattro I mass spectrometer (Micromass Inc.). The separation was obtained with a BDS Hypersil C18 column (25 cm × 4.6 mm i.d., 5 μm; Keystone Scientific Inc.), eluted with 2.5% CH_3CN in H_2O during the initial 10 min, and increasing amounts of CH_3CN up to 30% over the next 30 min. The flow was 1 mL/min, and a 20:1 split ratio was used before the sample entered the mass spectrometer. HRFABMS were obtained with a 70−4F instrument (University of Illinois, Mass Spectrometry Service).

IR spectra were obtained with a Perkin−Elmer 16 PC FT-IR instrument, and UV spectra with a Spectronic Genesys 2 spectrophotometer.

Insects, Extraction and Isolation. *P. versicolor* were collected at night in the vicinities of Ithaca (Tompkins County, NY), during summer (males and females) and early fall (larvae). Voucher specimens are deposited in the Cornell University Insect Collection. Females (120 specimens) were ground, defatted with hexane (3 × 50 mL), and extracted for 1 h with a MeOH−CH_2Cl_2 mixture (3:2, 4 × 50 mL). The residue was extracted one more time with the same solvent mixture (50 mL, 24 h). The combined extracts were evaporated under reduced pressure, yielding 271 mg of crude extract. A portion of the extract (90 mg) was dissolved in MeOH, filtered through a 0.2-μm syringe membrane filter, and fractionated by repeated HPLC injections (ca. 5 mg extract/injection). The fractions containing the compound of interest were then joined and purified in a single HPLC run, obtaining 1 mg of pure material. The samples of males and larvae that were examined were extracted with CH_3CN-H_2O (1:19).

***N*-Methylquinolinium 2-carboxylate (1):** mp 110 °C (dec); UV (CH_3OH) λ_{max} (log ϵ) 235 (4.35), 320 (sh) (3.97), 326 (3.99) nm; IR ν_{max} 3490, 1640, 1602, 1518, 1376, 1322, 1158, 1058, 880, 838, 774 cm^{-1}; NMR (Table 1); electrospray MS (Figure 1) 210 $[M + Na]^+$ (3), 189 (3), 188 $[M + H]^+$ (25), 166 (1), 160 (2), 146 (6), 145 (5), 144 (45), 143 (16), 142 (9), 140 (1), 130 (1), 129 (16), 128 (100), 127 (2), 117 (3), 116 (3), 115 (8), 103 (6), 102 (1), 101 (2); HRFABMS *m/z* 188.0712 (calcd for $C_{11}H_{10}NO_2$, 188.0712).

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