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January 21, 2003

Anthranilic acid reacts with chloroacetonitrile and *N, N*-diisopropylethylamine in DMF to yield 3-imino-2,3-dihydro-4,1-benzoxazepin-5(1*H*)-one (**1**) as the only reaction product. Also known as Ashley's fluorescer, compound **1** is fluorescent and binds to calcium carbonate, including the calcium carbonate-rich antennae of *Daphnia pulex*, the water flea.

Recently, we attempted to synthesize 2-[(cyanomethyl)-amino]benzoic acid from

anthranilic acid and chloroacetonitrile. Instead of generating the expected product, 3-imino-2,3-dihydro-4,1-benzo-xazepin-5(*1H*)-one (Ashley's fluorescer, **1**) was obtained in 66% yield as the only product formed (Scheme 1).

Scheme 1
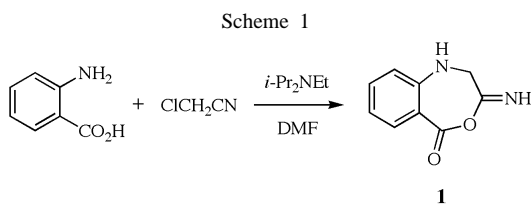
This is the first reported example of this heterocycle. The closest structures to **1** described in the literature are nitro- and aza-4,1-benzoxazepin-5(*1H*)-ones [1,2]. The iminooxy-carbonyl moiety in **1** is unique and is stable to the dilute aqueous acid and base conditions employed during its workup. The spontaneous 7-*exo-dig* cyclization to the benzofused ring in **1** is favored by Baldwin's ring-closure rules [3]. A plausible mechanism for the formation of **1** is shown (Scheme 2).

An unambiguous structural assignment for **1** was made based on elemental analysis, GC-MS, IR, ¹H-NMR, decoupled ¹³C-NMR, DEPT-135, and HMBC spectroscopy. GC-MS and elemental analysis gave $C_9H_8N_2O_2$ as the molecular formula. Since the IR spectrum demonstrated the absence of the nitrile peak between 2200-2300 cm⁻¹, it was clear that the expected 2-[(cyanomethyl)-amino]benzoic acid was not formed. Spectral data from ¹H-NMR, decoupled ¹³C-NMR, and DEPT-135 supported two possible structures, **1** and **2**, but could not distinguish them.
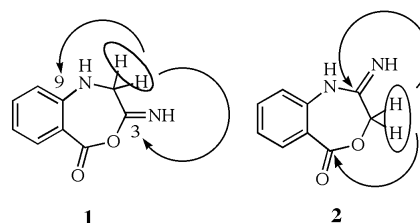
Figure 1. Long-Range ¹³C-¹H Correlations for Methylene Protons in **1** and **2**

However, two- and three-bond ¹H-¹³C correlations from HMBC made compound **1** the definitive structure (Fig. 1).

The methylene protons in compound **1** correlate to C-3 (δ166.3) and C-9 (δ115.6). Conversely, the same protons in compound **2** would correlate to *both* the carbons in C=N and C=O and would *not* correlate to any benzene ring carbons.

Increased structural rigidity in a molecule is known to increase fluorescence quantum yield [4]. Thus, under appropriate conditions, **1** may be more intensely fluorescent than anthranilic acid. Compound **1** also binds to solid calcium carbonate, which is a component of the antennae of *Daphnia pulex* [5]. Exposing *D. pulex* to 2.0 µM **1** in water caused the antennae to have pronounced fluorescence under fluorescence microscopy, highlighting the antennae more than the rest of the organism (Fig. 2c). In contrast, 2.0 µM anthranilic acid barely produced discernable fluorescence (Fig. 2b). Quantitative studies of the binding of **1** to calcium carbonate, other insoluble inorganic compounds of biological significance (e.g., calcium phosphate in bone), and biopolymers are presently underway.
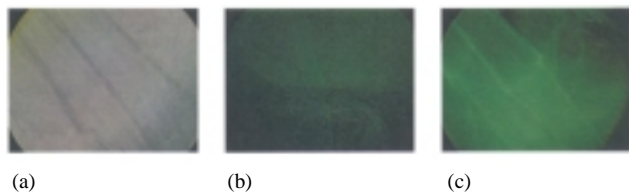
Scheme 2

(a)   (b)   (c)
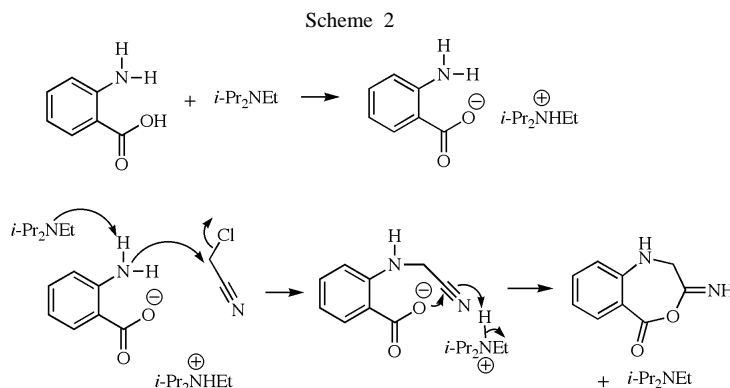
Figure 2. Microscopic pictures of *D. pulex* antennae: (a) under dissection microscope (40X); (b) under fluorescence microscope (40X) with 2.0 μM anthranilic acid; (c) under fluorescence microscope (40X) with 2.0 μM **1**.

EXPERIMENTAL

3-Imino-2,3-dihydro-4,1-benzoxazepin-5(*1H*)-one (**1**)

To a stirring solution of anthranilic acid (1.00 g, 7.3 mmol) in DMF (20 mL), *N,N*-diisopropylethylamine (2.55 mL, 7.3 mmol) and chloroacetonitrile (0.46 mL, 7.3 mmol) were added at room temperature. After 24 h, the reaction mixture was quenched with 200 mL 2 M hydrochloric acid, and then the pH was adjusted to pH 4 with 6 M sodium hydroxide. The reaction mixture was extracted with ethyl acetate (3 x 50 mL). The combined extract was dried over anhydrous magnesium sulfate and evaporated *in vacuo* to yield a crude white solid. Flash chromatography of the crude solid, using 3:1 hexanes:ethyl acetate as eluent, yielded **1** as a white, crystalline solid, 0.90 g (66%); mp 66.5-68.0 °C; ir (film): 3485, 3379, 1700, 1670 cm^{-1}; ^{1}H nmr (300 MHz, acetone-d$_6$): δ7.79 (dd, 1H, J = 8.2, 1.4); 7.29 (1H, m); 6.86 (1H, m); 6.61 (1H, m); 6.49 (s, 2H, N*H* and =N*H*); 5.13 (s, 2H, C*H$_2$*); ^{13}C nmr (75 MHz,): δ166.3 (s), 152.2 (s), 135.0 (d), 130.8 (d), 116.8 (d), 115.6(s), 115.4 (d), 107.5 (s), 48.5 (t); gc-ms (EI): *m/z* 176 (M$^+$).

Anal. Calcd. For C$_9$H$_8$N$_2$O$_2$: C, 61.36; H, 4.58; N, 15.90. Found: C, 61.45; H, 4.60; N, 15.70.

REFERENCES AND NOTES

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