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(+)-2,3-Dehydro-10-oxo-α-isosparteine in *Uresiphita reversalis* larvae fed on *Cytisus monspessulanus* leaves

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In honor of Professor Francis J. Schmitz's seventieth birthday.

Abstract

Quinolizidine alkaloids, found in the leaves of *Cytisus monspessulanus* L. (Leguminosae), were characterized in the cuticle of larvae of the pyralid moth *Uresiphita reversalis* (Lepidoptera: Pyralidae) when the latter were fed on this weed. By GC–MS analysis of the methanolic extracts of the cuticle, four quinolizidine alkaloids, *N*-methylcytisine, cytisine, aphylline and anagyrine, were identified as possible defense substances. In addition, the quinolizidine alkaloid, (+)-2,3-dehydro-10-oxo-α-isosparteine was characterized in both the insect and host plant.

Keywords: *Cytisus monspessulanus*; Leguminosae; French broom; *Uresiphita reversalis*; Pyralidae; Pyralid moth; Structural determination; Quinolizidine alkaloid; (+)-2,3-Dehydro-10-oxo-α-isosparteine

1. Introduction

The larvae of the pyralid moth *Uresiphita reversalis* (Lepidoptera: Pyralidae) are found peripherally on terminal branchlets of the exotic weed *Cytisus monspessulanus* L. (Leguminosae) (=*Genista monspessulana*, known as French broom) and actively feed on its leaves throughout the day. In previous insect–plant interaction studies, quinolizidine alkaloids were found to play an important role in the biology of the larvae of *U. reversalis* that feed on the leaves of *C. monspessulanus* L. (Montllor et al., 1990). During characterization of the alkaloids in the cuticle of *U. reversalis* larvae, a novel alkaloid was detected by GC–MS analysis in minute amount. The limited amount isolated from the insect source, however, was not enough to establish the final structure, and we attempted to isolate it from the host plant instead. In addition to the four known quinolizidine alkaloids, the target alkaloid was also isolated from the leaves of *C. monspessulanus* (Shibata et al., 1992). Wink et al. (1991) reported an alkaloid in their insect–host plant interaction study between *U. reversalis* and *C. monspessulanus* and suggested it as a dehydroapllyine derivative but the structure was not established. In this paper, the structural determination of this alkaloid as (+)-2,3-dehydro-10-oxo-α-isosparteine (**1**) is reported on the basis of spectroscopic methods (Fig. 1).

2. Results and discussion

The crude MeOH extract of the cuticle of *U. reversalis* larvae was subjected to GC–MS analysis, and it gave five peaks (***a***–***e***) as shown in Fig. 2. The main four peaks of ***a***, ***b***, ***d***, and ***e*** were *N*-methylcytisine (**2**), cytisine (**3**), aphylline (**4**), and anagyrine (**5**), respectively, as determined by direct comparison with authentic samples and mass spectroscopic analysis (Kinghorn and Balandrin, 1984). However, a minor peak ***c*** with a M^+ at 246 could not be identified by GC-MS analysis since its fragmentation pattern was not identical to any known quinolizidine alkaloids. Because of the limited availability of the extract from the insect source, the leaves of *C. monspessulanus* were used instead for identifying the unknown peak ***c***.

Chromatographic separation of the alkaloid fraction obtained from the MeOH extract of the fresh leaves of

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C. monspessulanus afforded **2**, together with an unknown alkaloid **1** in pure form. The molecular formula of **1** was determined as $C_{15}H_{22}N_2O$ by HRMS–EI, and ^1H and ^{13}C NMR spectroscopic analysis indicated that its structure is closely related to monspessulanine (**6**), previously isolated as the major constituent (25–35%) of the same plant (White, 1964). However, it should be noted that there were several differences in EI–MS spectra between **1** and aphyllidine (**7**) as a diastereomer of **6**. Hence, it seems that **1** is classified in a constitutional isomer of **6** (Sastry, 1972).

Assignments of all the protons and carbons for **1** were made through ^1H and ^{13}C NMR, and ^1H–^1H COSY experiments. The presence of an α,β-unsaturated lactam moiety was suggested by the absorption at 257 nm in the UV spectrum, and at 1670 (C=C) and 1650 (C=O) cm^{-1} in the IR spectrum. The ^1H NMR signals at $\delta = 7.74$ (H-2) and 4.89 (H-3) that correlated with each other in ^1H–^1H COSY spectra, and the ^{13}C NMR signals at $\delta = 166.9$ (C-10), 125.3 (C-2), and 107.5 (C-3) also indicated the presence of an α,β-unsaturated lactam system. Careful inspection of the ^1H–^1H COSY spectra revealed four cross peaks due to long range couplings between H-6 and H-17_{ax}, H-7 and H-9, H-8_{ax} and H-17_{eq}, and H-13_{eq} and H-15_{eq}, indicating that compound **1** has no less than four coplanar configurations (Kubo et al., 1984). The conformation of the C-ring is the chair form on account of the connection with a *gauche* position of H-7 and two H-17 with two small vicinal coupling constants about 2.5 Hz, and the presence of the long range coupling between H-8_{ax} and H-17_{eq}. The long range coupling between H-6 and H-17_{ax} suggests that the B-ring also possesses the chair form; therefore, the stereochemistry at H-6 is assigned as the β-configuration. The coupling constants between H-11 and two H-12 observed at about 13.5 and 3.0 Hz, and the long range coupling between H-13_{eq} and H-15_{eq} correspond to the chair form on the D-ring (Mikhova and Duddeck, 1999). The presence of evident downfield shifts at H-15_{eq} and H-17_{eq}, which come from the effect of the N-16 lone pair electrons, the similarity of ^{13}C NMR assignments between **1** and *epi*-applline, and the presence of Bohlmann bands at 2860–2770 cm^{-1} in the IR spectrum support the view that the stereochemistry of the quinolizidine ring system is (+)-α-isosparteine type (Bohlmann and Zeisberg, 1975). Consequently, the structure of **1** was confirmed as a novel alkaloid. The

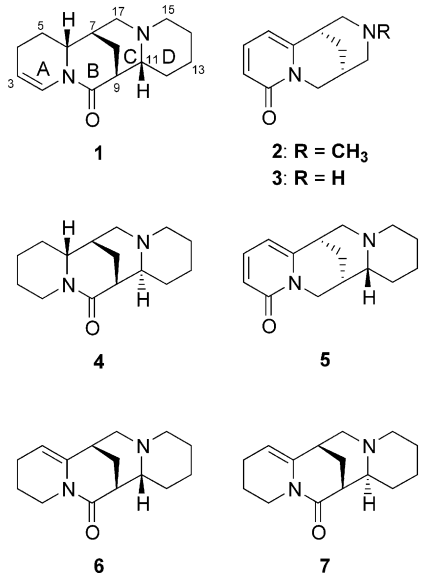

Fig. 1. Chemical structures of (+)-2,3-dehydro-10-oxo-α-isosparteine (**1**) and related quinolizidine alkaloids (**2**–**7**).

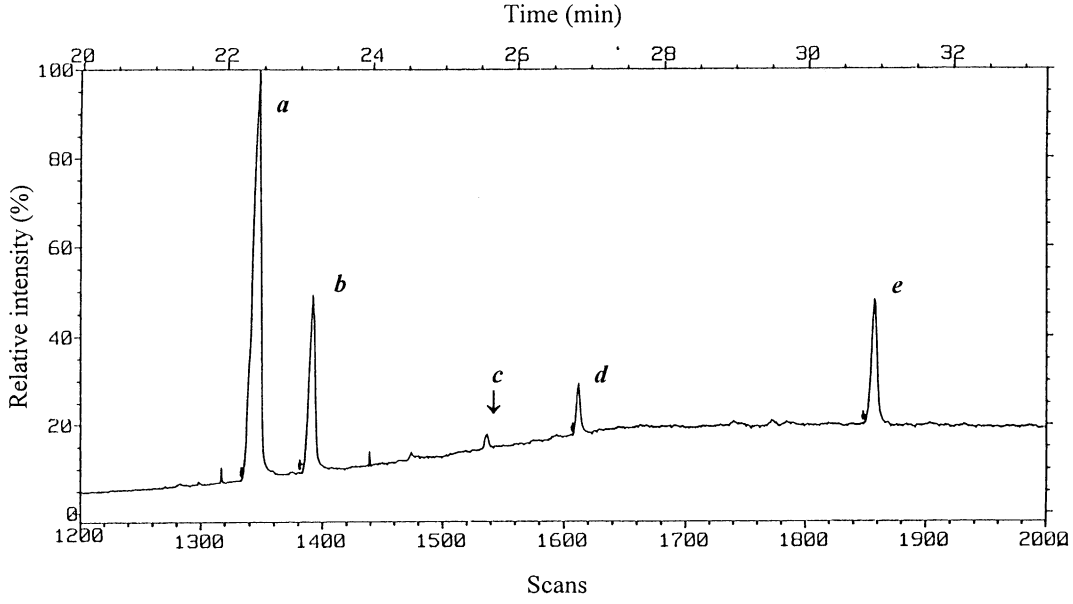

Fig. 2. Gas chromatographic separation of alkaloids in the cuticle of *U. reversalis* larvae.

result obtained suggests that *U. reversalis* larvae accumulate the alkaloids into their body by feeding on an alkaloid rich plant in order to prevent predation and microorganism infection (Saunders et al., 1992).

3. Experimental

3.1. General

IR spectra were obtained on a Perkin-Elmer model 1310 spectrometer in $CHCl_3$. UV spectra were acquired on a Hitachi 100–80 spectrometer in MeOH. ^1H and ^{13}C NMR spectra were recorded by a JEOL GX-400 spectrometer. HRMS–EI was measured with a Finnigan MAT-90 instrument (70 eV). The chromatograms were visualized by spraying with Dragendroff reagent. GC–MS spectra were recorded on a JEOL DX-300 mass spectrometer connecting to the gas chromatography equipped with 30 m 3% OV-17 capillary column. The GC operating condition employed were as follow: He as a carrier gas (18 ml/min); temp. program, 150 °C held for 1 min, increased 5 °C/min to 280 °C. Peak ***a*** (**2**); MS–EI, *m*/*z* (rel. int.): 204 [M^+] (39), 160 (8), 146 (8), 96 (4), 82 (4), 59 (5), 58 (100), 57 (3). Peak ***b*** (**3**); MS–EI, *m*/*z* (rel. int.): 190 [M^+] (87), 160 (24), 148 (37), 147 (99), 146 (100), 134 (22), 109 (21), 82 (18). Peak ***c*** (**1**); MS–EI, *m*/*z* (rel. int.): 246 [M^+] (100), 218 (20), 136 (70), 96 (52). Peak ***d*** (**4**); MS–EI, *m*/*z* (rel. int.): 248 [M^+] (47), 247 (52), 220 (49), 138 (29), 137 (46), 136 (100), 124 (23), 123 (25), 98 (34), 97 (42), 96 (36), 84 (26). Peak ***e*** (**5**); MS–EI, *m*/*z* (rel. int.): 244 [M^+] (53), 243 (12), 229 (5), 160 (11), 146 (16), 136 (14), 122 (8), 98 (100), 97 (11), 96 (11).

3.2. Plant and insect sources

The fresh leaves of *C. monspessulanus* were collected in UC Berkeley campus and extracted with MeOH at ambient temp. The cuticle of *U. reversalis* larvae fed on the leaves of *C. monspessulanus* was prepared as previously described (Montllor et al., 1990). Both materials were provided by Professor E.A. Bernays.

3.3. Extraction and isolation

Insect material was ovendried at 70 °C, ground in a mill to 40 mesh, and extracted in MeOH. The fresh leaves (200 g) of *C. monspessulanus* L. were extracted with MeOH. The dried extract was taken up in 1 M HCl, and partitioned with ether. The aqueous portion was then basified with ammonia to pH 10, extracted with $CHCl_3$. Consequently, 10.5 mg of the extract for GC–MS analysis was obtained from the 150 mg of insect cuticles. Likewise, 1.7 g of extract was obtained from the 200 g fresh leaves. The $CHCl_3$ extract of the leaves was subjected to silica gel chromatography eluted with 1–5% MeOH–$CHCl_3$ to give **1** (120 mg) and **2** (270 mg).

3.4. (+)-2,3-Dehydro-10-oxo-α-isosparteine (*1*)

Colorless needles, mp 98–103 °C, $[\alpha]_D^{26}$ +132° (EtOH; *c* 0.6). UV λ_{max}^{MeOH} nm (log ϵ): 257 (3.07). IR $\nu_{max}^{CHCl_3}$ cm^{-1}; 2860–2770 (Bohlmann bands), 1670, 1650. HRMS–EI (*m*/*z*): $[M]^+$ calc. for $C_{15}H_{22}N_2O$ 246.1738; found 246.1735. ^1H NMR (400 MHz, C_6D_6, δ): 1.07 (*qt*, *J* = 13.4, 3.9 Hz, 1H, H-13_{ax}), 1.30 (*m*, 1H, H-5_{eq}), 1.32 (*m*, 1H, H-7), 1.34 (*m*, 1H, H-14_{eq}), 1.40 (*m*, 1H, H-8_{eq}), 1.41 (*qt*, *J* = 13.4, 3.4 Hz, 1H, H-14_{ax}), 1.61 (*m*, 3H, H-8_{ax}, H-12_{eq}, H-13_{eq}), 1.68 (*dd*, *J* = 13.4, 2.7 Hz, 1H, H-11), 1.70 (*dt*, *J* = 2.7, 13.4 Hz, 1H, H-15_{ax}), 1.76 (*dd*, *J* = 11.5, 2.5 Hz, 1H, H-5_{ax}), 1.81 (*dd*, *J* = 12.0, 2.7 Hz, 1H, H-17_{ax}), 1.87–1.90 (*m*, 2H, H-4), 2.00 (*qd*, *J* = 13.4, 3.1 Hz, 1H, H-12_{ax}), 2.32 (*m*, 1H, H-9), 2.55 (*m*, 1H, H-15_{eq}), 2.67 (*dd*, *J* = 12.0, 2.4 Hz, 1H, H-17), 3.11 (*dd*, *J* = 11.5, 2.7 Hz, 1H, H-6), 4.89 (*m*, 1H, H-3), 7.74 (*d*, *J* = 8.4 Hz, 1H, H-2). ^{13}C NMR (100 MHz, C_6D_6, δ): 23.4 (*t*, C-4), 24.9 (*t*, C-13), 25.8 (*t*, C-14), 26.1 (*t*, C-5), 30.2 (*t*, C-8), 30.6, (*t*, C-12), 32.8 (*d*, C-7), 44.5 (*d*, C-9), 56.9 (*t*, C-15), 57.2 (*t*, C-17), 57.4 (*d*, C-6), 65.9 (*d*, C-11), 107.5 (*d*, C-3), 125.3 (*d*, C-2), 166.9 (*s*, C-10).

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