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Note

High-performance liquid chromatographic identification of simple β-carboline alkaloids in specimens of Heliconiini butterflies

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The simple β-carboline alkaloids have been identified in twenty-six plant families[1]. They are frequently used in biological research because of their activity as monoamine oxidase inhibitors[2]. Additionally, their activity extends to include being both comutagenic[3] and growth inhibitors of protozoans[4] and lepidopterans[5]. Because of their frequently small concentration in plant material, they have not been quantified in many cases where they are reported[1].

The principal method used for identification of simple β-carboline alkaloids in plant material has been thin-layer chromatography (TLC) utilizing their fluorescence characteristics for identification under long-wave ultraviolet (UV) light[6–9]. The fluorescence excitation maxima are at 375 nm and 368 nm for norharman (β-carboline, 9H-pyrido-[3,4-*b*]indole) and harman (1-methyl-9H-pyrido-[3,4-*b*]indole), respectively[9]. High-performance liquid chromatography (HPLC) coupled with UV absorbance has been used to identify some β-carbolines[10,11]. Two β-carbolines, harman and norharman, have been separated by HPLC and fluorescence detection using 270 nm excitation wavelength[3].

In this investigation, nanogram quantities of seven β-carboline alkaloids were separated on a reversed-phase C_{18} column and identified by means of coupling the HPLC with a fluorescence detector utilizing 370 nm excitation and 425 nm emission wavelengths. Samples of adult butterflies, which fed as larvae on plant material containing β-carboline alkaloids, were used in this study. Dried whole insect samples weighing as little as 100 mg (two or three specimens) were adequate for the identification of these alkaloids, even when the alkaloid concentration was low.

EXPERIMENTAL

Material

Dried samples of seven species of adult Heliconiini butterflies (Family: Nymphalidae) collected in the wild were used.

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Extraction procedure

Amounts of 100 mg to 1 g of dried and ground material were first extracted with methanol. The extract was evaporated to dryness under low pressure. A 10% hydrochloric acid solution was added, the solution filtered, basefied to pH 9, then extracted by partition with dichloromethane. The water fraction was basified to pH 12 with 10% sodium hydroxide and reextracted. The acid–base shake out and partition procedure was repeated. The dichloromethane fractions were combined and evaporated to dryness, and the residue redissolved in a known amount of spectral grade methanol for injection into the high-performance liquid chromatograph.

Standards and chemicals

Standard chemicals of the seven β-carbolines were obtained from Sigma. They were run through the experimental system to check for purity. All solvents in the extraction process were analytical grade. HPLC grade methanol was used with HPLC. Water was twice-distilled.

Apparatus

The HPLC solvent delivery system consisted of a Beckman Model 332 with a 420 Controller driving two 110A pumps. A Perkin-Elmer LC-10 Fluorescence detector used 370 nm excitation, 425 nm emission wavelengths. A Hewlett-Packard 3390A integrator reported retention times and peak areas.

HPLC

The stationary phase was a Varian prepacked analytical 10 μm, 30 cm × 4 mm, reversed-phase C_{18} column. The mobile phase began with methanol–water (65:35), and 0.01% triethylamine and continued in a varying gradient to methanol–water (90:10) at 2 ml/min (Fig. 1). Area determination of quantities of standard compounds were used to determine sample concentrations. Standard sample areas were determined over a 1–100 μg/ml range. Retention times were used to identify compounds.

Mass spectrometry (MS) and TLC

An elution peak identified as norharman by the HPLC method described above was subjected to verification by direct probe high resolution MS (VG Analytic 7070E with an ionizing potential of 70 eV. Accurate mass measurements were carried out by the peak matching method. Silica gel TLC coincident with standard samples of alkaloids was also used to confirm the presence of β-carboline alkaloids. The TLC solvent system used was dihloromethane–methanol–triethylamine (75:25:2).

RESULTS AND DISCUSSION

Fig. 1 represents the HPLC analysts of seven β-caroline standards and a representative chromatogram of *Heliconius erato petiverana*. Baseline separations were obtained except between 6-methoxy harman and harman. Detection limits were as low as 10 ng (harmine) to 0.1 ng (6-methoxyharman) in a 20 μl sample. Total separation time was less than 38 min, with a column recycling time of 10 min. The three major alkaloids identified and quantified by this method in adult specimens of Hel-

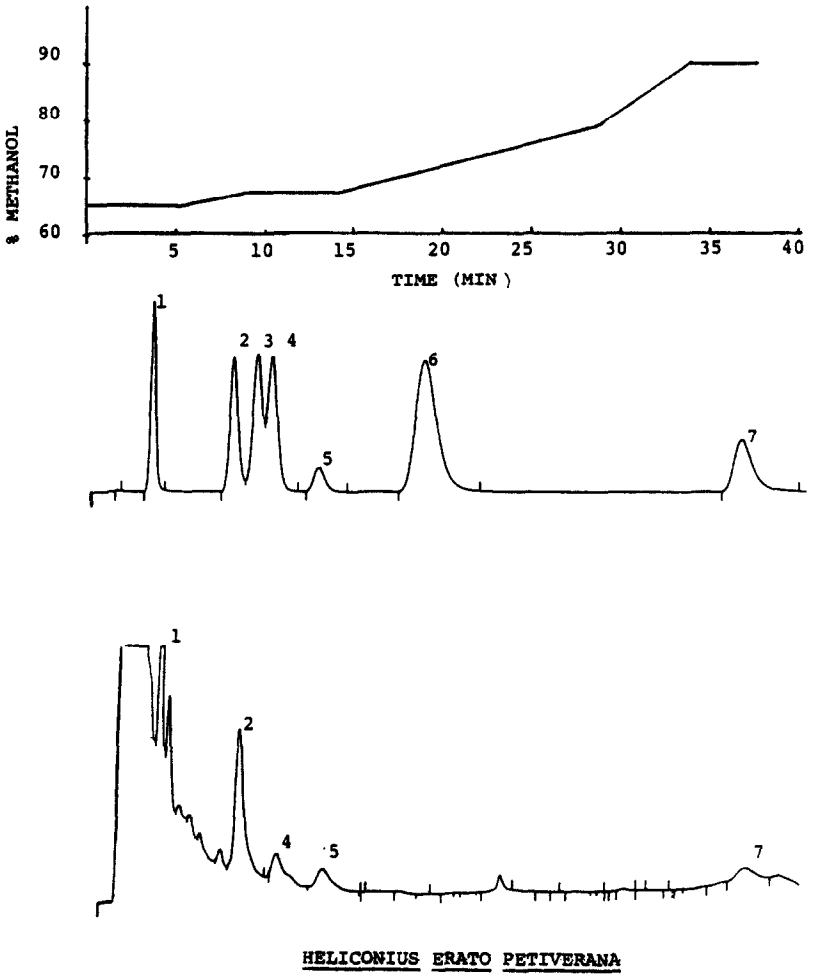

Fig. 1. HPLC analysis of β-carbolines and *Heliconius erato petiverana* extract showing liquid gradient. Peaks: 1 = harmol; 2 = norharman; 3 = 6-methoxy harman; 4 = harman; 5 = harmine; 6 = harmalol; 7 = harmaline.

TABLE I

ALKALOIDS IDENTIFIED IN FIELD COLLECTED SPECIMENS OF HELICONIINI

Species	*n*	*Picomol/individual (μg% dry weight)**			*Total*	*Collection site*
		1	*2*	*3*		
Acraea andromacha	10	13 (5)	106 (52.3)	+	119	Australia
Heliconius sara thamar	6	12 (0.5)	6 (3.3)	9 (6.0)	27	Belem
H. erato petiverana	20	70 (39.5)	14 (8.3)	96 (68.4)	180	UCI (C.R.)**
H. wallacei flavescens	3	+	9 (4.2)	+	9	Belem
H. ethilla eucoma	5	119 (52.3)	+ +	110 (61.2)	229	Belem
H. melpomene rosina	13	55 (22.5)	+	+	55	Pavo
H. Cydno galanthus	7	117 (32.5)	7 (2.2)	11 (4.0)	135	UCI (C.R.)

* 1 = Norharman; 2 = harman; 3 = harmine.

** UCI (C.R.): Colony specimens maintained at UCI, collected from Costa Rica.

iconiini are listed in Table I. Confirmation of peak identification was obtained by direct probe MS of a sample identified by this method as norharman. Peaks of mass 168.0692, 141.0574 and 114.0483 identify the molecular formulae $C_{11}H_8N_2$, $C_{10}H_7N$, and C_9H_8, and correspond to the molecular ion and previously reported major fragments of norharman[12]. TLC confirmed the presence of harman, 6-methoxy harman, harmine and harmaline in butterfly samples.

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REFERENCES

1 J. R. F. Allen and B. R. Holmstedt, *Phytochemistry*, 19 (1980) 1573.
2 G. H. Coates and B. Cox, *Eur. J. Pharmacol.*, 18 (1972) 284.
3 J. H. Peters, K. E. Mortelmans, E. J. Reist, C. C. Sigman, R. J. Spanggord and D. W. Thomas, *Environ. Mutagenesis*, 3 (1981) 639.
4 J. C. Cavin, S. M. Krassner and E. Rodriguez, *J. Ethnopharmacol.*, 19 (1987) 89.
5 J. C. Cavin and E. Rodriguez, *J. Chem. Ecol.*, 14(2) (1988) 475.
6 S. McLean and D. G. Murray, *Can. J. Chem.*, 50 (1972) 1478.
7 L. P. Bush and J. A. D. Jeffreys, *J. Chromatogr.* 111 (1975) 165.
8 V. J. Lutomski and B. Malek, *Planta Med.*, 27 (1975) 381.
9 I. A. Veliky, *Phytochemistry*, 11 (1972) 1405.
10 C. Nagalakshmi, A. S. Subbaraman, M. D. Pednekar and A. S. U. Choughuley, *Ind. J. Exp. Biol.*, 22 (1984) 84.
11 A. Al-Shamma, S. Drake, D. L. Flynn, L. A. Mitscher, Y. H. Park, G. S. R. Rao, A. Simpson, J. K. Swayze, T. Veysoglu and S. T.-S. Wu, *J. Nat. Prod.*, 44 (1981) 745.
12 R. T. Coutts, R. A. Locock and G. W. A. Slywka, *Org. Mass Spectrom.*, 3 (1970) 879.