Phytochemistry, Vol. 21, No. 5, pp. 1035–1037, 1982.
Printed in Great Britain.

0031-9422/82/051035-03$03.00/0

THE INDOLE ALKALOID, HYPAPHORINE AND *PTEROCARPUS* SEED PROTECTION

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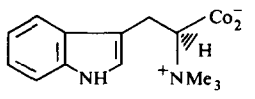
(*Received* 18 *August* 1981)

Key Word Index—*Pterocarpus officinalis*; Leguminosae; L-hypaphorine; indole alkaloid; *Liomys salvini*; rodent; feeding deterrence.

Abstract—L-Hypaphorine has been isolated as a major constituent of the seeds of *Pterocarpus officinalis*, seeds which are rejected as a food source by a wide variety of seed-eating rodents dwelling in the same habitat. Incorporation of the isolated hypaphorine into artificial diets of a small seed predator, *Liomys salvini* (a rodent) supports its role as a feeding deterrent.

INTRODUCTION

The large tree, *Pterocarpus officinalis* (Leguminosae), grows in natural pure stands of as much as 1 ha adjacent to the species-rich rainforests [1] of the Corcovado National Park in Costa Rica, a rainforest rich in seed-eating mammals (e.g. *Dasyprocta punctata, Aguti paca, Proechimys cayennensis, Tayassu tajacu, Tapirus bairdii, Heteromys desmarestianus, Oryzomys caliginosus, Sciurus granatensis, Microsciurus alfari* and several species of climbing rats). On approximately alternating years these stands of *P. officinalis* produce so many fruits that densities of 100/m^2 on the ground are commonplace. Each of the soft, indehiscent and quasi-wind-dispersed 4 cm diameter fruits contains a soft seed weighing 1–3 g (*ca* 60% dry wt). Despite this enormous abundance of readily available seeds, there is no sign whatsoever of seed predation by mammals. We have investigated these seeds and found that they contain *ca* 5% dry wt of the alkaloid L-hypaphorine (**1**). This alkaloid has now been shown to function as an effective mammalian feeding deterrent for the seed predator *Liomys salvini* (Heteromyidae) by incorporating L-hypaphorine into artificial diets.



1

RESULTS AND DISCUSSION

The water-soluble crystalline material isolated from the seeds of *P. officinalis* showed a strong UV maximum at 281 nm with shoulders at 289 and 274 nm, and the EI-MS (70 eV, 200°) gave an apparent molecular ion at *m/z* 187. The CI-MS (CH_4) positive ion spectra supported the molecular ion assignment with *m/z* 188.0712 ($C_{11}H_{10}NO_2$, calc. for 188.0727) and the negative ion spectra (CH_4/N_2O) showed evidence for chlorine attachment, *m/z* 222. Conflicting evidence appeared in the ^{1}H NMR (360 MHz) giving integrated intensities for at least 17 protons. The 9H singlet at δ 3.20 taken together with the broadened carbon signal at δ 50.1 and the intense, sharp IR (KBr) band at 1350 cm^{-1}, strongly supported the presence of a methylated quaternary amine [2], which is lost on volatilization in EI and CI mass spectrometry. Thermal fragmentation occurs to give a neutral fragment which undergoes protonation or chlorine attachment under chemical ionization conditions. Further assignments of the NMR data, taken together with optical rotation measurements, $[\alpha]_D^{22} + 102°$ (c = 0.05 m) have allowed the assignment of structure **1**, L-hypaphorine.

Hypaphorine was first isolated from seeds of *Erythrina hypaphorus* [3] and has been detected in several other papilionoid genera. High concentrations of alkaloids and non-protein amino acids are known to deter insect feeding [4–9] and it is interesting in this context that the seeds of the Costa Rican *Pterocarpus rohrii*, which lacks L-hypaphorine or other alkaloids, are attacked by the larvae of a weevil and a bruchid beetle [10]. Less is known about the ecological role these substances play as regards seed-eating mammals.

Liomys salvini, or spiny pocket mouse, was the only wild tropical rodent available for testing hypaphorine's role as a mammalian feeding deterrent (Table 1). *L. salvini* is a professional seed predator [11, 12] and consumes many species of tropical seeds during the year. It is the Costa Rican dry forest analogue to *Heteromys desmarestianus* [12, 13], the heteromyid seed-eating rodent that lives in the rainforest containing the stand of *Pterocarpus officinalis*. Five adult female wild-caught *L. salvini* weighing 39–50 g ($\bar{x}$ = 44.8 g; s.d. = 5.3) were placed on a diet of reconstituted laboratory rat chow (ground, adulterated, slightly moistened, pressed into balls, air

Table 1. Fate of *Liomys salvini* mice confined to a diet of pure lab. chow (December, July, 1980) or pure lab. chow adulterated to 5% hypaphorine (December 1980) (dry wt)

Mouse number	Sex	Caught weight (g)	Final weight (g)	%weight change	Number of days	Mouse fate	Absolute weight change per day
Fed 5% hypaphorine (December 1980)							
L.s. 70	♀	50	40	−20	3	Died	−3.3
L.s. 71	♀	45	37	−18	3	Died	−2.7
L.s. 72	♀	40	34	−18	3	Died	−2.0
L.s. 73	♀	39	33	−15	2	Died	−3.0
L.s. 75	♀	50	42	−16	3	Died	−2.7
Fed pure lab chow (December 1980)							
L.s. 90	♀	46	45	−2	5	Healthy	−0.8
L.s. 91	♂	59	59	0	5	Healthy	0
L.s. 93	♂	48	53	10	5	Healthy	1.0
L.s. 94	♂	67	65	−3	5	Healthy	−0.4
July 1980							
L.s. 37	♂	32	33	3	9	Healthy	0.1
L.s. 38	♀	40	41	3	7	Healthy	0.1
L.s. 39	♀	41	37	−10	7	Healthy	−0.6
L.s. 40	♂	59	59	0	7	Healthy	0
L.s. 41	♀	36	37	3	4	Healthy	0.3

dried) containing 5% dry wt pure L-hypaphorine extracted from seeds of *P. officinalis* (Table 1). The mice nibbled the adulterated laboratory chow and then refused to eat it. One died in the middle of the second day and four died in the middle of the third day, apparently of starvation. They lost 15–20% of their body weight while starving to death. Nine wild-caught adult control *L. salvini* (four females and five males, mean weight 47.6 g, s.d. = 11.8) were placed on a pure diet of reconstituted laboratory chow lacking L-hypaphorine for 4–9 days; their average weight at the end of this period was unchanged ($\bar{x} = 47.7$ g; s.d. = 11.6), as occurred in previous feeding trials of *L. salvini* on reconstituted laboratory chow [11–13].

There is no doubt that L-hypaphorine at concentrations equal to or somewhat less than that found in *P. officinalis* seeds is a feeding deterrent for at least one rodent that subsists in a large part on seeds and belongs to a family of rodents well-known as seed predators wherever they occur. However, there may also be other defensive compounds in *P. officinalis* seeds. That L-hypaphorine is serving more as a feeding deterrent than a direct toxicant is suggested by the outcome of preliminary feeding trials with two wild-caught black rats (*Rattus rattus*), using the reconstituted adulterated laboratory chow that was uneaten by the dead *L. salvini*. Like *L. salvini*, the black rats nibbled at the food and then refused to eat it, and began to lose weight at the same rate as they do with no food. However, on the third day of starvation, both rats began to eat the adulterated lab chow, became diarrhetic and continued to lose weight. By the sixth to seventh days, both rats stopped losing weight and had normal-appearing faeces, and were consuming as much of the adulterated lab chow as they normally consume of unadulterated laboratory chow. On the eighth day they escaped. In short, at least the black rat is capable of surviving for a few days on a diet rich in L-hypaphorine. However, its initial response to the adulterated laboratory chow suggests that only under circumstances of extreme starvation would the black rat choose *P. officinalis* seeds as food.

EXPERIMENTAL

Isolation. Newly fallen, living, but ungerminated *Pterocarpus officinalis* seeds were collected in Corcovado National Park in Costa Rica [1]. The finely ground oven-dried seeds (300 g) were extracted with 2.5 l. 80% MeOH and filtered. The filtrate, diluted to 50% MeOH with H_2O, was applied directly to a column (500 ml bed vol.) Amberlite IR-120, H^+ form. After washing with 1.5 l. H_2O, 2 N HCl and more H_2O, an alkaloid-positive material was eluted with 1 N NH_4OH. The ammonia was removed immediately under vacuum, and the residue, dissolved in H_2O, was applied to a column (150 ml) Amberlite CG-400, OH^- form. The alkaloid fraction passed through the column unbound, was treated with charcoal, concd and recrystallized from hot H_2O to yield 11 g material suitable for analysis: mp 246.5° with decomposition (lit. [14] 255° with decomposition); IR ν_{max}^{KBr} cm^{-1}: 1730, 1350; ^1H NMR (360 MHz, 1 mM, D_2O) δ 3.82 (α-H, *dd*, $J = 11.8$, 3.8 Hz), 3.39 (β-H, *dd*, $J = 13.2$, 3.8 Hz), 3.25 (β-H, *dd*, $J = 13.2$, 11.8 Hz), 7.13 (2H, *br s*), 7.56 (4H, *ddd*, $J = 8.0$, 1.3, 0.80 Hz), 7.07 (5H, *ddd*, $J = 8.0$, 7.8, 1.3 Hz), 7.15 (6H, *ddd*, $J = 8.2$, 7.8, 1.3 Hz), 7.40 (7H, *ddd*, $J = 8.2$, 1.3, 0.80 Hz), 3.20 [$N(Me)_3$, *s*]; ^{13}C NMR (20 MHz, 0.80 M in D_2O) δ 169.6 (CO_2^-), 124.8 (C7a), 123 (C-3a), 120.4 (C-2, C-5), 117.8 (C-7), 116.5 (C-4, C-6), 110.3 (C-3), 50.2 (α-C), 50.1 [$N(Me)_3$], 21.1 (β-C); EI–MS (70 eV, 200°), *m/z* (rel. int. 187 $[M-59]^+$, (76), 169 (13), 143 (100), 130 (51), 115 (82); CI–MS (CH_4) *m/z* (rel. int.) 202 (3), 188 (47), 144 (55), 88 (67), 61 (100), 60 (100); CI–MS (CH_4/N_2O) *m/z* (rel. int.) 222 $[M-59+Cl^-]^+$, 187 (80), 186 (36), 142 (20), 71 (100); Anal. C_7H_9NO; calc. C 68.27%, H 7.37%, N 11.37%; found C 68.04%, H 7.45%, N 11.43%.

Detection and estimation. Hypaphorine reacts positively with Dragendorff reagent (red) and Ehrlich reagent (slow blue). Seed hypaphorine concns in seeds were estimated at 5% by the intensity of the Dragendorff spot in methanolic HCl extracts.

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