EXPERIENTIA

Vol. 31 – Fasc. 4 Pag. 393 – 504 15. 4. 1975

SPECIALIA

Pyrrolizidine Alkaloids in *Parsonsia* Species (Family Apocynaceae) which Attract Danaid Butterflies

Male butterflies of the sub-family Danainae visit plants containing 1,2-dehydropyrrolizidine alkaloids to obtain the alkaloid precursors of their dihydropyrrolizine courtship pheromones[1,2]. The plant species known to be visited have been in the genera *Heliotropium, Tournefortia* and *Cynoglossum* (family Boraginaceae)[1,3–6], *Senecio* and *Eupatorium* (family Compositae) (T. E. PLISKE, personal communication) and *Crotalaria* (family Leguminoseae)[7,8] all well established as sources of 1,2-dehydropyrrolizidine alkaloids[9]. Recently it was reported[8,10] that Danaid butterflies are similarly attracted to *Parsonsia eucalyptophylla* (F. Muel.) and *Parsonsia straminea* ((R. Br.) F. Muel.) (family Apocynaceae) and male butterflies have also been observed to scratch the leaves of *P. straminea* and to feed on the exudates (G. SANKOWSKY, personal communication). We have examined these *Parsonsia* species and find them to contain the 1,2-dehydropyrrolizidine alkaloids lycopsamine, intermedine and/or indicine and the acetyl derivative of either intermedine or indicine, alkaloids previously found only in the Boraginaceae[9].

The family Apocynaceae is noted primarily for complex indole and steroidal alkaloids[11]. Pyrrolizidine alkaloids with a saturated pyrrolizidine ring bearing unusual substituent groups have been found in *Anodendron affine* Druce[12] and *Alafia multiflora* Stapf[13], both in the subfamily Echitoideae. A dihydropyrrolizine, closely related to the dihydropyrrolizines from male Danaid pheromone glands, has been isolated from extracts of *Fernaldia pandurata* Woodson (previously known as *Urechites karwinsky*)[14], also subfamily Echitoideae. Our present finding is, however, the first definite evidence that pyrrolizidine alkaloids of the biologically active 1,2-dehydro type occur in the Apocynaceae. *Parsonsia* and *Fernaldia* are in the same tribe (Parsonsieae) of the Echitoideae, thus strengthening the possibility that similar alkaloids are present in *Fernaldia*[15]. The fact that *Parsonsia* alkaloids are typical of the family Boraginaceae and the genus *Eupatorium* of the Compositae, where the diastereoisomers rinderine and echinatine occur[9], suggests an evolutionary sequence involving the relevant sections of the Apocynaceae, Boraginaceae and Compositae. This is in keeping with the broader proposals of TAKHTAJAN[16] and CRONQUIST[17] that the plant orders containing these families have a common origin.

The present finding also supports the hypothesis[15] that 1,2-dehydropyrrolizidine alkaloid-containing plants of the Boraginaceae visited by male Danaids, and the Danaids' cardenolide-containing larval food plants in the Apocynaceae and Asclepiadaceae, represent evolutionary branches of ancestral food plants which contained both 1,2-dehydropyrrolizidine alkaloids and cardenolides. In addition *Parsonsia* species occur widely in Queensland so it is now likely that they are the source of the lycopsamine found[2] unexpectedly in extracts of the hairpencils of 2 Danaid species, *Danaus hamatus hamatus* (Macleay) and *Euploea tulliolus tulliolus* (Fabricius), captured near Wallaville, Queensland. The previously known sources of lycopsamine, *Echium lycopsis* (L.) and *Amsinckia* species of the family Boraginaceae, probably do not occur near Wallaville[2].

The plants were assayed for alkaloid and alkaloid N-oxides by standard procedures[18]. The results are shown in the Table. The alkaloid extracts were then examined by combined gas chromatography-mass spectrometry, before and after conversion to methyl boronate derivatives, and by paper electrophoresis[19] in 3 different buffer systems (phosphate, pH 7.0; carbonate, pH 9.2; borate, pH 9.2)[19]. Comparison with authentic lycopsamine and its

[1] J. A. EDGAR, C. C. J. CULVENOR and G. S. ROBINSON, J. Aust. ent. Soc. *12*, 144 (1973).
[2] J. A. EDGAR and C. C. J. CULVENOR, Nature, Lond. *248*, 614 (1974).
[3] G. H. E. HOPKINS and P. A. BUXTON, Proc. ent. Soc., Lond. *1*, 35 (1927).
[4] E. B. POULTON, Proc. R. ent. Soc., Lond. (A) *11*, 94 (1936).
[5] R. A. LEVER, Proc. R. ent. Soc., Lond. (A) *11*, 95 (1936).
[6] W. BEEBE, Zoologica, N.Y. *40*, 27 (1955).
[7] C. MCCANN, J. Bombay nat. Hist. Soc. *51*, 752 (1952).
[8] A. F. ATKINS, Aust. ent. Mag. *1*, 78 (1974).
[9] L. B. BULL, C. C. J. CULVENOR and A. T. DICK, *The Pyrrolizidine Alkaloids* (North-Holland, Amsterdam 1968).
[10] A. F. ATKINS, Aust. ent. Mag., in press.
[11] R. HEGNAUER, *Chemotaxonomie der Pflanzen* (Birkhäuser Verlag, Basel und Stuttgart 1964), vol. 3, p. 127.
[12] K. SASAKI and Y. HIRATA, Tetrahedron *26*, 2119 (1970).
[13] M. PAIS, F. X. JARREAU, P. FOUCHÉ and R. GOUTAREL, *Annls pharm. fr. 29*, 57 (1971).
[14] J. BORGES DEL CASTILLO, A. G. ESPAÑA DE AGUIRRE, J. L. BRETÓN, A. G. GONZÁLEZ and J. TRUJILLO, Tetrahedron Lett. *1970*, 1219.
[15] J. A. EDGAR, C. C. J. CULVENOR and T. E. PLISKE, Nature, Lond. *250*, 646 (1974).
[16] A. TAKHTAJAN, *Flowering Plants, Origin and Dispersal* (Olivier and Boyd, Edinburgh 1969).
[17] A. CRONQUIST, *The Evolution and Classification of Flowering Plants* (Nelson, London 1968).
[18] C. C. J. CULVENOR and L. W. SMITH, Aust. J. Chem. *8*, 556 (1955).
[19] J. L. FRAHN, Aust. J. Chem. *22*, 1655 (1969).

Alkaloid content of *Parsonsia* species (% dry weight) [a]

Species	Total alkaloid (%)	Free alkaloid (%)	Alkaloid-N-oxide (%)
P. eucalyptophylla	0.844	0.048	0.796
P. straminea	0.030	0.008	0.022

[a] Method of CULVENOR and SMITH[18].

methyl boronate derivative established the presence of lycopsamine in the extracts of both species. In addition both contained a lycopsamine isomer, either intermedine or indicine. Intermedine and indicine differ only in the sign of the optical rotation of their esterifying acids ((+)-trachelanthic and (−)-trachelanthic respectively) and complete identification of this component must await isolation and more complete characterisation. The mass spectrum of the 3rd component found in the *P. eucalyptophylla* extract indicated that it was a monoacetyl derivative of lycopsamine or indicine/intermedine where the acetyl group was on one of the esterifying acid hydroxyls. Hydrolysis of the *P. eucalyptophylla* alkaloids under mild conditions[20] resulted in loss of the acetyl derivative peak in the gas chromatogram and a corresponding increase in the size of the intermedine/indicine peak suggesting that the third component is acetylintermedine or acetylindicine[21].

Résumé. Il a été montré que deux espèces de *Parsonsia* (famille Apocynaceae), qui attirent les papillons mâles de la sous-famille Danainae, contiennent des alcaloïdes du type 1,2-dehydropyrrolizidine, trouvés jusqu'à présent seulement chez les Boraginaceae.

J. A. EDGAR and C. C. J. CULVENOR

CSIRO Division of Animal Health, Animal Health Research Laboratory, Private Bag No. 1, P.O., Parkville (Victoria, 3052, Australia), 3 December 1974.

[20] A. R. MATTOCKS, J. chem. Soc. (C) *1967*, 329.

[21] Acknowledgments. We thank Dr. J. L. FRAHN for the paper electrophoresis results and P. COCKRUM and N. ANDERTON for assistance.

Structure of Uperolein, a Physalaemin-Like Endecapeptide Occurring in the Skin of *Uperoleia rugosa* and *Uperoleia marmorata*[1]

Since 1966 it has been recognized that methanol extracts of the skin of the Australian leptodactylid frogs *Uperoleia rugosa* and *Uperoleia marmorata* contain large amounts of a new peptide (uperolein) possessing a biological activity very similar to that of physalaemin[2].

Uperolein has now been isolated in a pure form and shown to be an endecapeptide with the following sequence:

Pyr-Pro-Asp-Pro-Asn-Ala-Phe-Tyr-Gly-Leu-Met-NH_2

Because uperolein differs from physalaemin only with respect to 2 amino acid residues, it may be considered as a Pro[2]-Ala[6]-physalaemin.

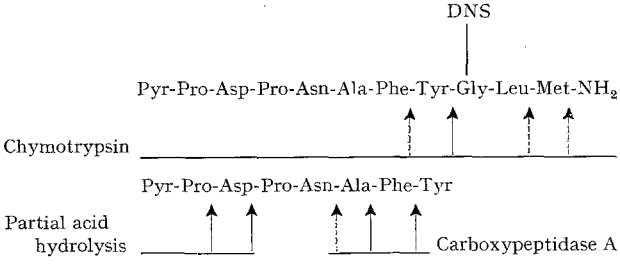
Isolation procedure. 1081 specimens of *Uperoleia rugosa*, captured in Queensland and New South Wales during the period 1970–1973 yielded 59.8 g of dried skins. The different batches of skins were extracted twice with 80% methanol immediately after their arrival in our laboratory.

An aliquot of extract corresponding to 50 g dried skin was evaporated to dryness and the residue dissolved in 95% ethanol. The liquid was passed through a column of 170 g of alkaline alumina which was then eluted with ethanol-water mixtures (each of 200 ml) containing decreasing concentrations of ethanol. The physalaemin-like activity appeared in the 60% ethanol eluate and the active fraction was found to be almost free of contaminants by chromatographic and electrophoretic criteria.

Accordingly, the material obtained from the alumina column was used directly for the structural analysis, or it was further purified by preparative electrophoresis.

On high voltage electrophoresis on paper, the active spot was found to possess no mobility towards the cathode at acidic pH, denoting the absence of positive charges due to free amino groups or to basic amino acids, and the mobility of a negatively charged peptide in neutral medium ($E_{5.8}$ = 0.25 Glu). The spot was positive to chlorine and to the reagents for tyrosine (Pauly and α-nitroso-β-naphthol reagents) but it was negative to ninhydrin confirming that the N-terminal group was not free.

Structure. The structure of uperolein was deduced by sequential analysis of the fragments obtained by digestion with chymotrypsin, followed, as shown below, by digestion with carboxypeptidase A, partial acid hydrolysis, and dansylation (DNS).



[1] Supported in part by grants from the Consiglio Nazionale delle Ricerche, Roma.

[2] V. ERSPAMER, G. DE CARO and R. ENDEAN, Experientia *22*, 738 (1966).