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.

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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.

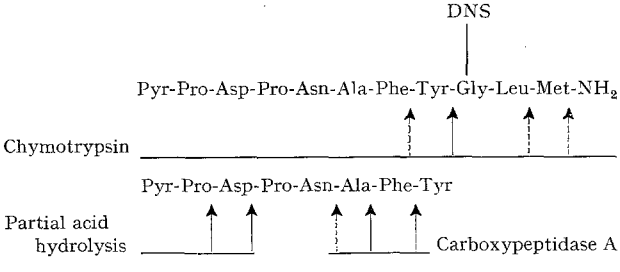
Isolation procedure. 1081 specimens of *Uperoleia rugosa*, captured in Queensland and New South Wales during the period 1970–1973 yielded 59.8 g of dried skins. The different batches of skins were extracted twice with 80% methanol immediately after their arrival in our laboratory.

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).



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Uperolein was demonstrated to occur also in the 60% ethanol eluate obtained from an alumina column loaded with an extract of the skin of *Uperoleia marmorata* (130 adult specimens = 8 g dried skin).

In addition to uperolein, *Uperoleia marmorata* skin contained another physalaemin-like peptide, closely related to, but not identical with, uperolein, which emerged mainly in the 30% ethanol eluate. On high voltage electrophoresis, it showed the same electrical mobility as physalaemin ($E_{1\cdot8}$ = 0.43 Glu; no mobility at pH 5.8).

A physalaemin-like peptide different from uperolein also appeared in 40 and 30% ethanol eluates of the *U. rugosa* extract, but it is not known whether this second peptide is identical with the second peptide found in the 30% ethanol eluate of *U. marmorata*. Elucidation of the structures of these peptides is in progress.

Riassunto. Gli estratti metanolici di pelle degli anfibi australiani *Uperoleia rugosa* e *Uperoleia marmorata* contengono due o tre polipeptidi fisaleminosimili. Il più importante di essi, l'uperoleina, è stato isolato allo stato di purezza e all'analisi sequenziale è risultato essere l'endecapeptide Pro^2-Ala^6-fisalemina.

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Inhibition of Catecholamine and 5-Hydroxytryptamine Induced Enzyme Secretion from the Guinea-Pig Submandibular Gland by 2-Bromo-D-Lysergic Acid Diethylamide[1]

5-Hydroxytryptamine (5-HT, serotonin) has recently been shown to enhance enzyme secretion from the rat and rabbit parotid glands[2,3], as well as from the guinea-pig submandibular gland[4,5]. In the latter gland, the secretory effect of 5-HT can be abolished by both α- (phenoxybenzamine) and β- (propranolol) adrenergic blocking agents in vitro[5]. Furthermore, the amylase secretory response of 5-HT, injected i.v. into rabbits, can be partly (45%) inhibited by initial treatment of the animals with propranolol[3]. The exact mode of action, however, of this biogenic monoamine, as well as the presence of a specific 5-HT receptor in mammalian salivary glands has not been established. In addition, 5-HT markedly enhances fluid secretion from the abdominal salivary gland of the blowfly, and it has been suggested that this effect is mediated via cyclic AMP[6,7].

2-bromo-D-lysergic acid diethylamide (BOL 148) is an effective 5-HT-blocker in various tissues[8–11]. In the present investigation, the effect of BOL 148 on 5-HT, dopamine, noradrenaline, adrenaline and dibutyryl cyclic AMP-theophylline-induced peroxidase and amylase secretion from the guinea-pig submandibular gland was studied in vitro.

Materials and methods. Male guinea-pigs, 3 months of age, weighing roughly 300 g, were used. The animals were starved overnight before being anesthetized by an i.p. injection of sodium pentobarbital (Mebumal®, ACO, Sweden). The submandibular glands of 2 animals were rapidly excised and extraglandular tissue removed under a stereomicroscope. The glands were cut into small fragments and randomly distributed among the incubation vessels. The basal medium used was a Krebs-Ringer bicarbonate buffer (pH 7.4) supplemented with pyruvate, glutamate and fumarate[12] and also containing albumin

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Table I. Peroxidase release. In vitro effects of BOL 148 on the secretion of peroxidase from guinea-pig submandibular gland

Secretagogues	No. of experiments	Non-stimulated secretion	Control (stimulated secretion)	Test Secretagogue + BOL 148 (2×10^{-4} *M*)	Difference (test-control)
DBcAMP (1 m*M*) + theophylline (5 m*M*)	5	1.6 ± 0.14	10.7 ± 3.28	14.8 ± 4.07	+ 4.1 ± 3.80
Noradrenaline (10^{-5} *M*)	5	1.1 ± 0.14	14.9 ± 3.10	0.84 ± 0.15	−14.0 ± 3.16[c]
Adrenaline (10^{-5} *M*)	5	1.3 ± 0.20	6.2 ± 1.17	0.74 ± 0.09	− 5.4 ± 1.21[c]
5-HT (10^{-4} *M*)	5	1.1 ± 0.14	9.6 ± 1.07	1.8 ± 0.35	− 7.8 ± 1.13[b]
Dopamine (10^{-4} *M*)	4	1.7 ± 0.11	17.8 ± 2.03	1.8 ± 0.46	−16.1 ± 2.17[b]

After a preincubation period of 30 min at 37 °C the specimens were incubated in a supplemented Krebs bicarbonate buffer for 60 min with listed concentrations of secretagogues. BOL 148 (2×10^{-4} *M*) was present during both the preincubation and incubation periods. The enzyme release is expressed as percentage of the total peroxidase activity in tissue and medium. Mean values (%) ± S.E.M. for indicated number of experiments. [a] $P < 0.001$; [b] $p < 0.01$; [c] $p < 0.05$.