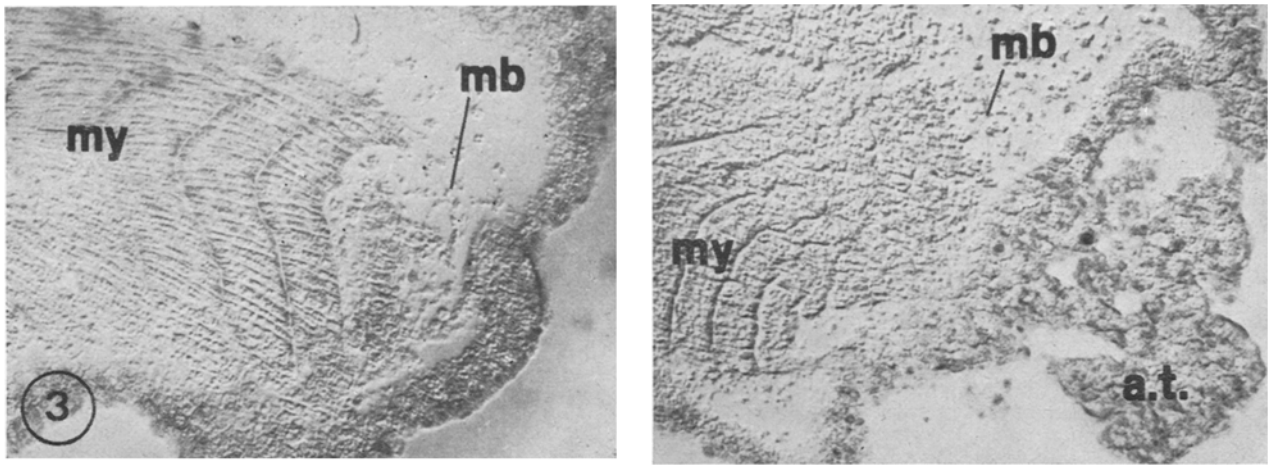

Fig. 3. Internal healing is also seen in the form of notochordal cap for instance (day 3).

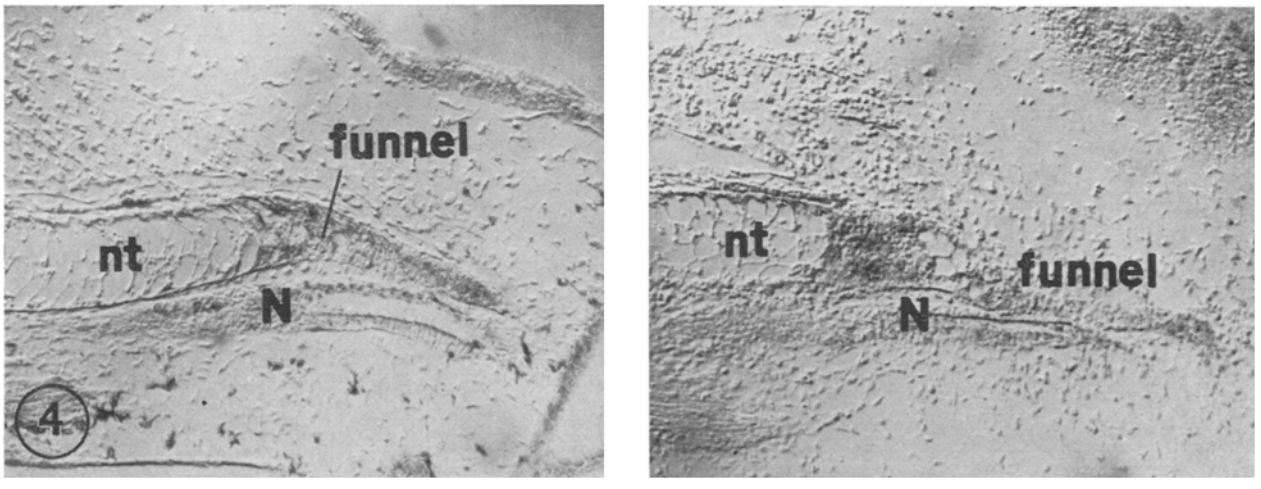

Fig. 4. Even myotomes disorganize into loose masses of myoblast-like cells (day 5).

logical functions are perhaps sequestered and left unaffected by the elimination of the paternal genome. One of these must then be a general propensity to grow and regenerate. The apparent normal regeneration in haploid tadpoles can be related to the significant finding that certain organelles (mitochondria and cilia) have retained the same dimensions in *n* as in *2n* cells of *Xenopus* larvae[7].

Admittedly, it is not possible to follow regeneration of haploid tails to its completion because of the precocious death of the tadpoles. But it is clear that much information regarding nucleocytoplasmic interactions in general and haploidy specifically will be gained by a continual appraisal of haploid function and dysfunction. In this manner it should become possible to catalogue and separate those functions which are affected by the haploid condition from those which are not as has already been done with respect to water regulation during haploidy[8].

[7] H. FOX and L. HAMILTON, J. Embryol. exp. Morph. *26*, 81 (1971).
[8] L. HAMILTON and P. H. TUFT, J. Embryol. exp. Morph. *28*, 449 (1972).

9,10-Dihydroergotamine: Production of Antibodies and Radioimmunoassay

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Summary. Antibodies against 9,10-dihydroergotamine (DHE) were produced by immunizing rabbits with a conjugate of 6-nor-6-carboxymethyl-9,10-dihydroergotamine and bovine serum albumin. A highly specific and sensitive radioimmunoassay for DHE has been developed.

Complete separation of antibody-bound hapten from free hapten is a prerequisite for an efficient radioimmunoassay. The successful use of the coated charcoal technique[2] allowed us to realize a simple but highly sensitive and specific test for an ergot alkaloid. Owing to the low plasma concentration of 9,10-dihydroergotamine (DHE) in the blood after an oral dose of 2.5 mg DHE mesylate, it is not possible to determine the concentration of the active compound in plasma samples by physico-chemical methods. For this reason, a radioimmunoassay has been

Table I. Blood levels (pmol/ml plasma) of 9,10-dihydroergotamine (DHE) in rhesus monkeys after gavage of 2 tablets of Dihydergot®, each containing 2.5 mg DHE mesylate

Time after gavage (h)	No. of animal				
	801	713	805	807	804
1/2	1.08	0.86	0.48	0.52	0.58
1	0.56	0.86	0.58	0.44	0.96
1 1/2	0.44	0.58	0.44	0.30	0.52
2	0.30	0.48	0.30	0.26	0.50
2 1/2	0.12	0.36	0.26	0.16	0.28
3	0	0.22	0.28	—	—
4	0	0	0.28	—	—
6	0	0	0	—	—
8	0	0	0	—	—

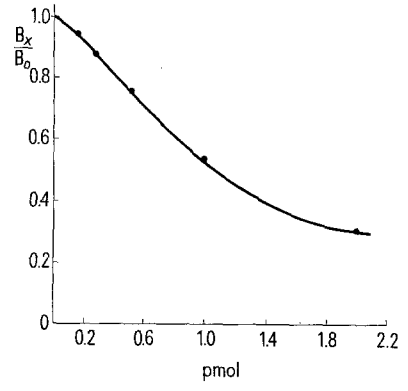

Fig. 1a. Radioimmunoassay of 9,10-dihydroergotamine: typical standard curve. The antiserum was employed at a final dilution of 1:640.

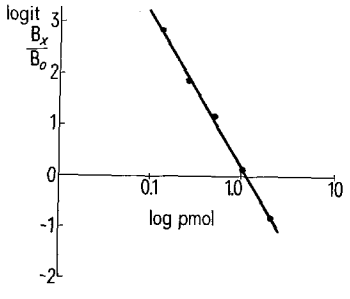

Fig. 1b. Logit plot calculated for the standard curve shown in Figure 1a, logit $y = 0.150–3.091 \log x$, $r = 0.998$.

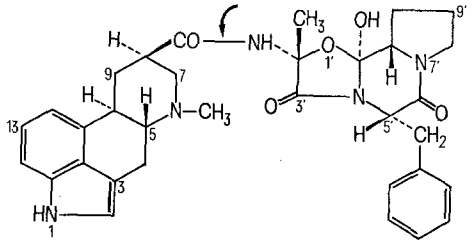

Fig. 2. Structural formula and absolute configuration of 9,10-dihydroergotamine. Possible metabolites are derived from the two parts of the molecule resulting from cleavage of the CO-NH bond (see arrow).

developed, by means of which it has proved possible to estimate the unchanged compound in the plasma.

Antibodies were produced in rabbits by immunizing them with a conjugate of 9,10-dihydroergotamine and bovine serum albumin (DHE-BSA) and with a conjugate of 9,10-dihydroergotamine and poly-L-lysine (DHE-pLYS). 6-Nor-6-carboxymethyl-9,10-dihydroergotamine (100 mg) (T. FEHR, unpublished) was dissolved in 1.5 ml 50% aqueous pyridine, to which 1.5 ml of an aqueous solution of bovin serum albumin (100 mg) was added. This mixture was agitated by magnetic stirring. An aqueous solution (0.5 ml) of 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide hydrochloride (175 mg)[3] was then added. The resulting solution was stirred for a further 2 h and left to stand overnight in the dark at room temperature. The product was then successively dialyzed against 2-l volumes of 50%, 25% and 10% aqueous pyridine and finally against deionized water at 4 °C. The freeze-dried DHE-BSA conjugate was practically insoluble in water. A conjugate of DHE and poly-L-lysine hydrobromide prepared in similar fashion, and having a molecular weight of approximately 100,000, was soluble in water. It was possible to differentiate DHE-*p*LYS from free DHE by thin layer chromatography, using the van Urk reagent[4].

One group of 3 male rabbits (silver-fawn) were immunized with DHE-BSA and another group of 3 rabbits with DHE -*p*LYS in the following way: an emulsion was prepared consisting of 7 mg conjugate in 1.4 ml 0.1 *M* sodium bicarbonate, 1.4 ml 2% aluminium hydroxide Cγ suspension and 4.2 ml Freund's complete adjuvant. 1 ml of emulsion was administered to each animal in divided doses in the back (i.c.) and in the hindlegs (i.m.). 3 weeks later the animals were given a booster injection. After 1 and after 2 weeks, blood samples were taken from the marginal vein of the ear. The titre (dilution of the antiserum in the incubation mixture affording 50% binding of labelled hapten) rose to 1:1,000 in the animals immunized with DHE-BSA, but in those immunized with DHE-*p*LYS, the maximum titre attained was 1:120. All 6 rabbits reacted to the immunogens.

The radioimmuno-assay was carried out with a 1:640 dilution of the antiserum produced with DHE-BSA. A solution of 9,10-dihydroergotamine-[13-^{3}H]-base (^{3}H-DHE) (E. SCHREIER, unpublished) (5 ng/ml; 30.4 mCi/mg) and a stock solution of DHE (20 pmol/ml) were freshly prepared daily with pH 7.5 buffer (8.77 g NaCl, 1.65 g $NaH_2PO_4 \cdot 2\,H_2O$, 0.62 g H_3BO_3, 0.96 g $Na_2B_4O_7 \cdot 10\,H_2O$, 0.2 g NaN_3 and 25 ml normal rabbit serum made up to 1 l with deionized water). 0.3 ml saline was added to 0.5 ml of sample (plasma or serum) and it was incubated with 0.1 ml antiserum for 10 min at room temperature. 0.1 ml ^{3}H-DHE solution was then mixed with the sample which was left to stand at room temperature for 10 min. Free hapten was separated from bound with 0.5 ml of a 1% suspension of charcoal in pH 7.5 buffer. The sample was immediately mixed, left at room temperature for 5 min and centrifuged for 10 min at 1500 × *g*. Aliquots (1.0 ml) of the supernatant containing antibody bound

[1] We are greatly indebted to Dr. T. FEHR for the synthesis of 6-nor-6-carboxymethyl-9,10-dihydroergotamine, and to Dr. E. SCHREIER for providing us with 9,10-dihydroergotamine-[13-^{3}H]-base of high specific activity.

[2] V. HERBERT, K.-S. LAU, CH. W. GOTTLIEB and S. J. BLEICHER, J. clin. Endocr. *25*, 1375 (1965).

[3] T. L. GOODFRIEND, L. LEVINE and G. D. FASMAN, Science *144*, 1344 (1964).

[4] E. STAHL, *Dünnschichtchromatographie*, 2nd edn. (Springer Verlag, Berlin 1967), p. 432 and 825, No. 68.

Table II. Within-assay scatter for DHE radioimmunoassay standard curve produced in plasma from rhesus monkey

pmol DHE mesylate ± SD
0.125 ± 0.024
0.25 ± 0.069
0.50 ± 0.082
1.0 ± 0.066
2.0 ± 0.127

tracer were then measured by β-counting after the addition of a scintillator, e.g. Instagel®, Scintisol® complete. Detection was limited to 0.125 pmol DHE per sample (0.5 ml of plasma or serum), corresponding to a ratio (B_x/B_0) of 0.94. The S-shaped standard curve obtained (Figure 1a) was linearized and the best fit was found with the aid of the logit function (Figure 1b).

The antiserum to DHE was also tested for possible cross-reaction with metabolites of DHE. The metabolism of DHE is complex, but it is known that the molecule undergoes cleavage into 2 moieties (Figure 2) thus giving rise to two series of metabolites, derivatives of 9,10-dihydrolysergic acid and derivatives of the peptide moiety (J. R. KIECHEL, unpublished). 50 pmol of 9,10-dihydrolysergic acid failed to give a cross-reaction. With 6 pmol peptide moiety as homologous cyclolcarbonic acid[5], the ratio B_x/B_0 is 0.8, i.e. at this concentration 20% of the cyclolcarbonic acid is bound to antibody.

A practical investigation was then carried out with Dihydergot® in rhesus monkeys. The aim of the investigation was to estimate the unchanged drug in the blood after an oral dose of 2 tablets, each containing 2.5 mg DHE mesylate. The blood levels measured after various intervals of time are indicated in Table I in pmol/ml plasma. The within-assay scatter (± SD) at the 95% confidence level (each value based on 4 observations) is given in Table II.

The radioimmunoassay described is a sensitive and specific test for the detection of 9,10-dihydroergotamine in plasma or serum. The steric specificity of the antibody is such that metabolites derived from the two moieties of the molecule (Figure 2) do not cross-react within the range investigated. It has thus proved possible to determine intact ergot alkaloid in pmol quantities, thus permitting direct determination of pharmacokinetic parameters, such as half-life of elimination, with reference to unchanged drug.

[5] A. HOFMANN, *Die Mutterkornalkaloide* (F. Enke Verlag, Stuttgart 1964), p. 88.

Dermatologically Active Sesquiterpene Lactones in Trichomes of *Parthenium hysterophorus* L. (Compositae)

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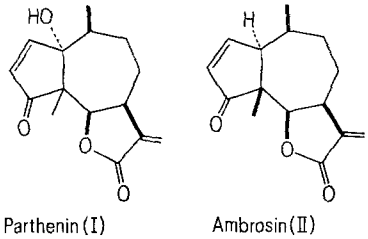
Summary. Scanning electron microscopy of the leaf surface, phyllaries and achene-complex of *Parthenium hysterophorus* L. showed the presence of 4 types of glandular and non-glandular trichomes. Chemical analysis established the presence of sesquiterpene lactones in the trichomes that cause eczematous dermatitis.

It has been shown recently that a common cause of allergic contact dermatitis in man is the sesquiterpene lactones found commonly in members of the Compositae[4]. The cosmopolitan weed, *Parthenium hysterophorus* L. is currently the cause of a serious outbreak of allergic eczematous dermatitis in parts of India, e.g., Poona, where it was introduced in 1956 from the Americas[5]. The allergenic compounds in this aggressive weed are the pseudoguaianolides, parthenin (I) and ambrosin (II), which are also found in other genera of the Compositae such as *Iva*, *Ambrosia* and *Hymenoclea*[6–10] (Figure 1).



Parthenin (I) Ambrosin (II)

Fig. 1. Allergenic Sesquiterpene Lactones.

[1] Acknowledgments. We thank Dr. GARRY COLE, University of Texas, for the use of the AMR-1000 Scanning electron microscope and JUDY STEVENSON for technical assistance. The work at the University of Texas was supported by the National Science Foundation (Grant No. BMS 71-01088) and the Robert A. Welch Foundation (Grant No. F-130).

[2] Dermatology Research, Shaughnessy Hospital, Vancouver (B. C., Canada).

[3] Department of Botany, University of British Columbia, Vancouver (B. C., Canada).

[4] J. MITCHELL, G. DUPUIS and T. A. GEISSMAN, Br. J. Derm. *87*, 235 (1972).

[5] A. LONKAR, J. MITCHELL and C. D. CALNAN, Trans. St. John's Hosp. Derm. Soc. *60*, 43 (1974).

[6] E. RODRÍGUEZ, Chemistry and Distribution of Sesquiterpene Lactones, Flavonoids in *Parthenium* (Compositae): Systematic and and Ecological Implications. Unpublished Ph. D. Thesis, University of Texas, Austin (1975).

[7] E. RODRÍGUEZ, H. YOSHIOKA and T. J. MABRY, Phytochemistry *10*, 1145 (1971).

[8] W. HERZ, in *Recent Advances in Phytochemistry* (Eds. T. J. MABRY, R. E. ALSTON and V. C. RUNECKLES; Appleton-Century Crofts, New York 1968), vol. 1, p. 229.

[9] W. HERZ, in *Chemistry in Botanical Classification*. Nobel Symposia 25 (Eds. G. BENDZ and J. SANTESSON; Academic Press, New York 1973).

[10] F. P. TORIBRO and T. A. GEISSMAN, Phytochemistry *7*, 1623 (1968).