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ERGOT-TYPE ALKALOIDS IN VEGETATIVE TISSUE OF *RIVEA CORYMBOSA* (L.) HALL. f.*

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Abstract—The ergot alkaloids, ergine and isoergine, were found in the leaf and stem but not in the root of *Rivea corymbosa*, which had been grown in a greenhouse. The amount per plant increased with time reaching a maxima of 0·027 and 0·012 per cent dry weight in the leaf and stem, respectively, after approximately 9 months' growth.

INTRODUCTION

THE seed "Ololiuqui", of the tropical convolvulaceous plant *Rivea corymbosa*, has been used as a narcotic by the Aztecs and neighbouring Indians since pre-Hispanic times.[1] It has been shown recently that these seeds contain at least five alkaloids of the ergot and clavine type, including ergine (*iso*lysergic acid amide), isoergine (lysergic acid amide), chanoclavine and elymoclavine.[2,3,4] The psychotoxic properties of ergine and isoergine have been described by Solmes[5] who reported that the effective dose of isoergine was about ten times that of lysergic acid diethylamide. Only one reference has been made to the possible use of portions of the plant other than the seed for narcotic purposes.[1] The sporadic attention given to the "Ololiuqui" plant in the past 25 yr has been devoted entirely to studies of the seed.[2,3,4,6,7,8] This tropical plant will grow in temperate regions, but has not been reported, or observed by us, to flower there. Thus it could be a regional source of these alkaloids only if other portions of the plant contained these substances. This communication reports the detection of the ergot-type alkaloids in the leaf and stem, but not the root, of *Rivea corymbosa* (L.) Hall. f.

RESULTS

Location of alkaloids

The alkaloid contents of root, stem and leaves of plants grown for various periods of time in the greenhouse were estimated by the van Urk assay of single ether extractions of freeze-dried tissues (Table 1). The values shown in the table representing the amount of

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[2] A. HOFMANN and A. TSCHERTER, *Experientia* **16**, 414 (1960).
[3] A. HOFMANN and A. CERLETTI, *Deut. med. Wochschr.* **86**, 885 (1961).
[4] A. HOFMANN, *Planta Med.* **9**, 354 (1961).
[5] H. SOLMES, *J. Clin. Exptl. Psychopathol. and Quart. Rev. Psychiat. Neurol.* **17**, 429 (1956).
[6] C. G. SANTESSON, *Arch. Pharm.* **275**, 532 (1937).
[7] H. OSMOND, *J. Mental Sci.* **101**, 526 (1955).
[8] W. A. TABER and R. A. HEACOCK, *Can. J. Microbiol.* **8**, 137 (1962).

alkaloid per plant are low, since a single extraction did not remove all of the alkaloid. These values demonstrate, however, that the amount of alkaloid per plant increased with age and that the root did not contain a detectable quantity of ergot-type alkaloids. Further, the amount of alkaloid per plant does not increase beyond that initially present in the seed[8] from which it grew until the plant is well beyond the cotyledonous stage. The rapid accu-

TABLE 1. DISTRIBUTION OF ALKALOID IN PLANTS OF VARYING AGE

Plant at harvest			μg Alkaloid per plant found in plant parts*		
Age (days)	Height (cm)	Dry weight (mg)	Root	Stem	Leaf
7	3	5	0	5	8
13	5	13	0	3	3
20	8	17	0	2	2
103	38	5,200	0	60	53

* All values are low since they were obtained by a single ether extraction of the freeze-dried plant material. See Table 2 for total alkaloid of mature plants.

mulation of alkaloid during the late growth phase (Table 1) is also characteristic of the fungus *Claviceps purpurea* (Fr.) Tul. Under conditions suitable for *in vitro* production of ergot-type alkaloids by this fungus, virtually all of the alkaloid is produced in a few days, and once production commences the yield can double in 48 hr.[9]

Total alkaloid

The total alkaloid content of plant parts was obtained by exhaustive extraction of freeze-dried tissues with ethyl acetate and with ether, and assaying by both the van Urk[9] and the fluorometric[10] methods (Table 2). The two methods of assay give similar but not

TABLE 2. TOTAL ALKALOID CONTENT OF PLANT PARTS*

Exhaustive extraction with	Age of plants (days)	Dry weight of plants (g)	Per cent dry weight content		
			Leaf	Stem	Root
Ethyl acetate	180	96†	0·016	0·010	0
Ether	277	62‡	0·027	0·012	0

* Estimated by van Urk assay.
† Grown by sub-irrigation in gravel culture.
‡ Grown in soil in flower pot.

identical values. The difference is due to the fact that while these alkaloids act identically on a molar basis with the van Urk reagent, their fluorescence maxima and intensities differ somewhat.[10] The fluorescent assay data are not given here. The maximum content for mature leaf and stem noted to date is 0·027 and 0·012 per cent dry weight, respectively (Table 2). The time required for maximal production can be expected to vary with the growth conditions. The root did not contain a detectable amount, although mature roots however, do contain substances giving a purple–red color with van Urk reagent. The

[9] W. A. TABER and L. C. VINING, *Can. J. Microbiol.* 4, 611 (1958).
[10] L. C. VINING and W. A. TABER, *Can. J. Microbiol.* 5, 441 (1959).

concentration of ergot and clavine alkaloids in the leaves is not as high as that of the seeds which contain approximately 0·06 per cent on a fresh weight basis.[8] Since the plant produces but one seed per flower, however, there would probably be at least as much total alkaloid in the leaf and stem of a plant as in its seeds.

Paper and thin layer chromatography, employing several standard ergot and clavine alkaloids, indicated that ergine and isoergine together with at least two other unidentified products showing typical blue fluorescence of lysergic acid derivatives were present in the extracts. Supporting the thin layer and paper chromatographic data are: (*a*) the rapid reaction of the material with the van Urk reagent to give the typical blue color, and (*b*) the fluorescence maxima.[10] Tryptophane and indole acetic acid, which gave a bluish color with the reagent after some delay, were not present, since extraction was with alkaline ether. Chromatography also showed these to be absent. Indole and tryptamine do not give a blue color.

The other members of the Convolvulaceae are currently being screened for the presence of ergot-type alkaloids and preliminary colorimetric and chromatographic data indicate that these alkaloids are present in seed, leaf and stem of commercial morning glories.

EXPERIMENTAL

Growth and harvest of plants

Plants were grown from Cuban seed[8] in a greenhouse at 25° in gravel culture by sub-irrigation with modified Hoagland's solution.[11] They were illuminated with 18 hr of combined natural and fluorescent light daily. Plants were harvested at intervals, separated into leaf, stem and root portions, freeze-dried, and powdered in a Wiley mill.

Alkaloid assay

Portions to be assayed colorimetrically with the van Urk reagent were extracted either with ether after being made alkaline with NH_4OH, or with ethyl acetate, after being made alkaline with $NaHCO_3$. In both cases, the alkaloid was back-extracted into 0·2 N H_2SO_4[10,12] and then reacted with van Urk reagent. The intensity of blue color was measured at 550 mμ within 2 min of mixing sample and reagent.[8] Samples of plant to be assayed by the fluorometirc method[10] were extracted with an aqueous 1% tartaric acid solution. The values shown in Table 1 were obtained using a single alkaline-ether extraction of plant tissue and by assaying with van Urk reagent. The values shown in Table 2 were obtained by exhaustive extraction with both ether and ethyl acetate and by assay with van Urk reagent. The fluorescence assay agreed in general with the colorimetric assay and the results are not reported herein.

Paper chromatography

The solution of alkaloid in 1% tartaric acid, as obtained from the extraction used for the fluorometric assay procedure, was neutralized with excess $NaHCO_3$ and extracted into chloroform. The mixture of alkaloids in the chloroform extract was examined by paper chromatography using the formamide–chloroform system[12] and by thin layer chromatography on silica plates with 8% chloroform in methanol as running solvent (cf. Hofmann[2]).

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