Phytochemistry, 1963, Vol. 2, pp. 65 to 70. Pergamon Press Ltd. Printed in England

CLAVINE AND LYSERGIC ACID ALKALOIDS IN VARIETIES OF MORNING GLORY*

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(*Received* 16 *July* 1962)

Abstract—The seeds of a number of commercially available varieties of Morning Glory (*Ipomoea* and *Convolvulus* sp.) have been found to contain clavine and lysergic acid alkaloids. Using thin layer and paper chromatographic methods, ergine, isoergine, ergometrine, ergometrinine, elymoclavine, penniclavine, and chanoclavine have been tentatively identified. Not all varieties of seed tested contained these alkaloids; one which contained a substantial amount also contained alkaloid in the leaves and stem of the mature plant.

INTRODUCTION

"OLOLIUQUI" and "Badoh Negro", the seeds of the convolvulaceous *Rivea corymbosa* (L.) Hall. f. and *Ipomoea violacea* (L.), respectively, have long been used by the Aztec Indians to attain a state of mind suitable for divination during traditional religious ceremonies.[1,2] The active principle has only recently been isolated by Hofmann and co-workers; lysergic acid amide (isoergine), a known psychotomimetic, is present and is accompanied in the seed by ergine, elymoclavine, chanoclavine and lysergol as well as other related substances in smaller amounts.[3,4,5] Subsequent studies in our laboratories[6,7] showed that the alkaloids are present in the microbially sterile embryo, and also in the leaf and stem, but not the root, of the mature plant. From this evidence it was considered reasonably certain that the alkaloids are a true metabolic product of the plant and not of an invading microbial parasite or contaminant.

The discovery of clavine and lysergic acid alkaloids in higher plants is of considerable interest since compounds of this type have previously been encountered only in a limited number of fungi, notably *Claviceps* sp. Their presence in two tropical representatives of the family Convolvulaceae prompted us to examine some ornamental varieties of morning glory. These are derived from the genus *Ipomoea* and perhaps also *Convolvulus*, although the origin of these ornamentals is obscure.[8] In this paper the detection of small amounts of several clavine and lysergic acid alkaloids in certain commercially available varieties is described.

* Issued as N.R.C. No. 7182.

[1] R. E. A. SCHULTES, *A contribution to our knowledge of* Rivea corymbosa. *The narcotic ololiuqui of the Aztecs.* Botanical Museum of Harvard University, Cambridge, Mass. (1941).
[2] A. HOFMANN, *J. Exptl. Med. Sci.* **5**, 31 (1961).
[3] A. HOFMANN and A. TSCHERTER, *Experientia* **16**, 414 (1960).
[4] A. HOFMANN and A. CERLETTI, *Deut. Med. Wochschr.* **86**, 885 (1961).
[5] A. HOFMANN, *Planta Med.* **9**, 354 (1961).
[6] W. A. TABER and R. A. HEACOCK, *Can. J. Microbiol.* **8**, 137 (1962).
[7] W. A. TABER and R. A. HEACOCK, unpublished.
[8] A. N. KEMPF, Bodger Seeds Limited, El Monte, California (personal communication).

RESULTS

The alkaloid extracted from alkali-wetted seeds of morning glory (variety "Pearly Gates") by organic solvents using four different procedures ((*a*) to (*d*), see Experimental) and assayed by the van Urk reagent[9,10] was 0·067, 0·067, 0·075 and 0·088 per cent; direct extraction with aqueous ethanol and purification on ion-exchange column (procedure (*e*)) gave 0·120 per cent. The content varied somewhat with the lot of seed. Another lot of this variety contained 0·042 per cent (Table 1) when extracted by procedure (*b*). The first three

TABLE 1. ALKALOID CONTENT OF CONVOLVULACEOUS SEEDS*

Package of common name†	% Fresh weight
Heavenly Blue (California)	0·024
Pearly Gates (California)	0·042
Ipomoea rubro-caerulea praecox	0·057
Convolvulus sp, Royal Blue	0·018
Crimson Rambler (California)	nil
Scarlet O'Hara (California)	nil
Convolvulus tricolor, Royal Marine	0·021
Convolvulus mauritanicus	0·009
Ipomoea cardinalis	nil
Ipomoea hybrida, Darling	0·016
Ipomoea purpurea	0·001
Convolvulus tricolor, Cambridge Blue	0·011
Ipomoea sp, Pearly Gates	0·024
Convolvulus, Lavender Rosette	0·014
Ipomoea, Scarlet O'Hara	0·014
Convolvulus tricolor	0·012
Rivea corymbosa, Ololiuqui (Cuba)	0·04

* Extracted with ammoniacal ether. The values are expressed as ergometrine equivalents using the van Urk reagent.[9,10]

† All seeds obtained from England except as noted.

procedures were considered to give equivalent yields, and the one using ether (*b*) was selected for use in a survey of different seeds (Table 1). All but three of the varieties examined contained detectable amounts of van Urk-reacting substances.[9,10] On the other hand, a large number of other non-convolvulaceous seeds gave negative results including those of mustard (*Brassica alba*), Swedish rape (*Brassica napus*), Golden rape (*Brassica napus*), Polish "rape" (*Brassica campestris*), broad bean (*Vicia faba*), stringless bean (*Phaseolus vulgaris*), soy bean (*Glycine hispida*), castor bean (*Ricinus communis*), "Cuthbertson Exhibition" sweet pea (*Lathyrus odoratus*), "Floribunda" sweet pea (*Lathyrus odoratus*), hemp seed (*Cannabis sativa*), buckwheat (*Fagopyrum esculentum*), grapefruit (*Citrus maxima*), safflower (*Carthamus tinctorius*), sunflower (*Helianthus annuus*), Double African marigold (*Tagetes* sp.), "Peppermint Stick" zinnia (*Zinnia* sp.), Dahlia Flowered zinnia (*Zinnia* sp.), "Golden Gleam" nasturtium (*Tropaeolum* sp.), "Flanders Field Pierrot" poppy (*Papaver dubium*), California poppy (*Eschscholtzia californica*), Half-sugar Rose mangel (*Beta vulgaris*), and "Peerless" watermelon (*Citrullus vulgaris*).

The morning glory variety "Pearly Gates" was grown in a greenhouse, and plants 37 and 48 days of age examined for alkaloids. The leaf and stem portions of these young plants contained 24 and 16 μg, respectively, per plant on the 37th day, and 23 and 25 μg, respectively, on the 48th day. None was found in the roots. The results are comparable

[9] W. A. TABER and L. C. VINING, *Can. J. Microbiol.* 3, 55 (1957).
[10] L. C. VINING and W. A. TABER, *Can. J. Microbiol.* 5, 441 (1959).

to those found for *Rivea corymbosa* where the young plant contained little alkaloid. Mature plants of *R. corymbosa*, however, contained up to 10 mg alkaloid.[7]

A qualitative comparison by thin-layer chromatography of the extracts obtained by direct extraction and ion-exchange purification from "Pearly Gates", *Ipomoea rubro-caerulea praecox*, "Heavenly Blue", and "Ololiuqui" showed little difference in the types of alkaloid present. Quantitative differences were apparent, however, and a comparison of the approximate composition of "Pearly Gates" and "Ololiuqui" (Table 2) illustrates the

TABLE 2. COMPARISON OF THE ALKALOID CONTENT OF SEEDS OF "OLOLIUQUI" AND OF THE MORNING GLORY "PEARLY GATES"

R_f of thin layer zone*	Fluorescence	Standards†	% total alkaloid recovered‡	
			Pearly Gates	Ololiuqui
Origin and 0·01	·	Tryptophan	65·8	16·2
0·03	—	Chanoclavine	10·7	6·9
0·10	+	—	0·9	1·6
0·15	—	—	0·9	1·0
0·19	—	Elymoclavine	1·1	2·7
0·23	+	Isoergine and penniclavine	6·5	33·7
0·28	+	Ergometrine	2·7	3·7
0·31	—	—	1·1	1·3
0·33	+	—	1·1	4·8
0·45	+	Ergometrinine	0·9	3·3
0·55	+	Ergine	4·7	18·3
0·64	+	—	3·0	4·2
0·85	+	—	0·7	2·3

* All substances give a positive van Urk reaction.
† See Table 3.
‡ The values refer to the % total alkaloid recovered from the chromatogram. The amount of alkaloid recovered from "Pearly Gates" and "Ololiuqui" was 79 and 67% respectively, of that applied to the chromatogram as determined on a separate extract.

differences which can occur. The recovery of alkaloid was 79 and 67 per cent, respectively, of the total, applied to the chromatogram, the per cent values recorded in Table 2 refer to the composition of the alkaloid accounted for. "Ololiuqui" contains relatively more of the substance suspected to be the psychotomimetic alkaloid, isoergine. This could account for the fact that, whereas numerous references to the psychotoxic properties of "Ololiuqui" exist, as far as the authors are aware there are no such references to the common morning glory.

From a comparison of their mobility on thin layer chromatograms with those of available standards, a number of van Urk-positive zones from the extracts could be tentatively identified. Additional evidence, provided by re-running the substance eluted from a thin layer chromatogram zone in at least two paper chromatographic systems, has strengthened these identifications (Table 3), but conclusive proof must await their separation and isolation in pure state. Such evidence has been provided by Hofmann and his group for the presence of ergine, isoergine, chanoclavine and elymoclavine in "Ololiuqui".[3,4]

One of the more interesting results is the apparent presence of ergometrine and ergometrinine in both "Ololiuqui" and varieties of morning glory seeds. These two lysergic acid derivatives have hitherto been encountered only in *Claviceps* species. Compounds F and G (Table 3) may also be substituted amides of lysergic acid and *iso*lysergic acid,

respectively. Isomerization in alkaline methanol showed that F gave rise to a second zone chromatographically identical with G and vice versa. In solvent system No. 3, F and G have the same *Rf* values as ergotamine and ergosinine, respectively. It is probable that one of the remaining unidentified zones is due to lysergol, which Hofmann and co-workers found in "Ololiuqui". Unfortunately a reference sample of this alkaloid was not available to us.

TABLE 3. *Rf* VALUES OF EXTRACTS FROM ZONES SCRAPED FROM THIN LAYER PLATES; "PEARLY GATES" VARIETY

Rf of thin layer zone	Solvent system*						Identified with
	1	2	3	4	5	6	
Origin to 0·01	—	0·02	—	0·50	0·60	0·73	Tryptophan
0·01	—	0·10	—	0·69	0·91	0·73	Compound A
0·03	—	0·18	—	0·69	0·91	0·73	Chanoclavine
0·10	0·24	—	0·42	0·51	—	—	Compound B
0·15	0·47	—	0·33	0·48	0·21	—	Compound C
0·19	0·33	0·63	—	0·52	—	0·67	Elymoclavine
0·23	0·18	0·56	0	0·52	0·68	0·50	Penniclavine
	0·29	0·56	0·02	0·52	0·60	0·50	Isoergine
0·28	0·29	0·69	—	0·60	—	0·52	Ergometrine
0·31	0·63	0·70	0·13	0·86	—	—	Compound D
0·33	0·63	0·70	0·18	0·52	—	—	Compound E
0·45	0·71	—	0·04	0·69	—	0·69	Ergometrinine
0·55	0·71	0·83	0·07	—	—	0 59	Ergine
0·64	1·00	—	0·30	0·85	—	—	Compound F
00·85	1·00	—	0·70	0·91	—	—	Compound G

* The solvent systems are listed below:

1. Chloroform on paper treated with formamide adjusted to pH 9 with NH_4OH.
2. The lower phase of chloroform-pyridine-formamide (9 : 1 : 5) as the mobile phase on paper treated with formamide of pH 9·0.
3. Benzene on paper treated with formamide of pH 6·0 (adjusted with formic acid).
4. Ethyl acetate–acetic acid–water (14 : 3 : 3).
5. *n*-Butanol–acetic acid–water (12 : 3 : 5).
6. Isopropyl ether–acetone–water (2 : 4 : 1) on paper treated with 1 per cent methanolic solution of tartaric acid.

Little information is available on the nature of compound A, which represents a majority of the van Urk-positive material extracted from "Pearly Gates" (Tables 2 and 3). It is likely to be acidic since it is not extracted into organic solvents from seeds wetted with alkali. The lack of fluorescence, somewhat more lipophylic behavior than tryptophan, and immediate blue color given with the van Urk reagent (tryptophan and tryptamine react slowly and give a green zone on chromatograms) suggest that it might be a new clavine alkaloid lacking a double bond conjugated to the indole ring.

EXPERIMENTAL

Morning glory seeds were purchased from Steele Briggs Seeds Limited, Winnipeg, local stores, and Thomspon and Morgan (Ipswich) Limited, England. The former company reported that their seed was supplied by growers in southern California. *Rivea corymbosa* seeds were purchased from the Atkins Garden and Research Laboratory, Cienfuegos, Cuba. The non-convolvulaceous seeds were purchased from local merchants.

Estimation of alkaloids

The seeds were powdered in a Wiley mill, and weighed samples assayed for ergoline derivatives by a colorimetric procedure using the van Urk reagent as described elsewhere.[9,10]

Certain samples were assayed also by a fluorometric procedure.[10] The two methods gave, in general, comparable results although neither was considered highly accurate.

Extraction methods

The efficiency of five methods for the complete extraction of alkaloids from 1·5 g lots of seed was compared as follows:

(*a*) Extraction of a wetted mixture of powdered seed and sodium bicarbonate with ethyl acetate. The solvent fraction was taken to dryness and the residue taken up in a mixture of ether and either 0·1 N H_2SO_4 or 1% tartaric acid, the alkaloids being present in the lower layer.

(*b*) Extracting the powdered seeds wetted with 10% ammonium hydroxide with ether, and then as for (*a*).

(*c*) A procedure developed by Alexander and Banes which employs a mixture of methanol and chloroform and concentrated ammonia.[11]

(*d*) As for (*a*) but extracting in a Soxhlet for up to 10 hr.

(*e*) An ethanol extract of powdered seed was applied to a column (1·4 × 12 cm) of Dowex 50W × 2(H^+) and the resin washed with fresh 80% ethanol.[9] Weakly basic material was then eluted with 3% solution of ammonia in 80% ethanol. The eluate was evaporated to dryness and taken up in dilute acid.

(*f*) The ammoniacal–acetone method described by Cromwell.[12] This method was unsuccessful when applied to seeds.

Thin layer chromatography

The alkaloids in the aqueous acid solutions from procedures (*a*) to (*d*) inclusive were extracted into chloroform after neutralizing with excess sodium bicarbonate. The chloroform was removed *in vacuo*, and the residue redissolved in 0·2 ml of ethanol. When procedure (*e*) was used the eluate, after evaporation to dryness, was redissolved in ethanol.

Qualitative comparisons of samples were made by applying 15 μl of each solution as a single spot to a plate (4 × 10 in.) of silica gel G (Merck). Comparable amounts of available standards were placed alongside the unknown for direct comparison of R_f values, and the chromatogram was developed with a chloroform-methanol (17 : 3) mixture. The positions of fluorescent zones were marked, the plate then sprayed with van Urk reagent, and the appearance of the zones noted.

Estimation of the approximate amount of alkaloid in each zone was carried out in the following way: the whole extract from 1·5 g of seed was applied as a line at the origin, leaving approximately $\frac{3}{4}$ in. at each edge, and aliquots from a separate extraction* placed as single spots along side. After the chromatogram had been developed the central area was masked, and the outer strips sprayed with van Urk reagent. Using these, and the fluorescence of the zones in the central area as guides, the adsorbent containing appropriate zones was scraped from the plate into test tubes. To each tube was added 0·1 N H_2SO_4 and the suspension stirred. Aliquots were removed, treated with the van Urk reagent and filtered before measurement of the optical density of the solution.

* The extract from another batch of seed was estimated colorimetrically for total alkaloids in order to determine the per cent recovery.

[11] T. G. ALEXANDER and D. BANES, *J. Pharm. Sci.* **50**, 201 (1961).
[12] B. T. CROMWELL, *Modern methods of plant analysis* (Edited by K. PAECH and M. V. TRACEY), Vol. IV, p. 413. Springer-Verlag, Berlin (1955).

When the substance from each zone was required for examination of its mobility in paper chromatographic systems, the adsorbent was extracted with methanol. An exception was made with the zone at and near the origin, when 90% methanol containing 5% acetic acid was used as the eluting agent. The eluate was concentrated under a stream of nitrogen at 50°C.

Paper chromatography

See footnote of Table 3.

Acknowledgements—Grateful acknowledgement is extended to Mr. D. W. Mansfield and Miss M. J. A. Gates, of the Prairie Regional Laboratory, and to Miss M. E. Mahon, of the Psychiatric Research Unit, for excellent technical assistance.