Changes in Ergoline Alkaloids in Seeds During Ontogeny of *Ipomoea violacea*

By K. GENEST

Ipomoea violacea L. (*Convolvulaceae*) (heavenly blue) plants were grown under greenhouse conditions. Seed samples were taken following the tenth day of fertilization until maturity was reached. Their content of total alkaloids, lysergic acid amide, isolysergic acid amide, and clavine alkaloids was determined. The alkaloid values were highest (about 0.1 per cent of dry seed) during the early stages of seed development. Chanoclavine was the most abundant alkaloid in the immature seed. With increasing maturity the lysergic acid amide/chanoclavine ratio increased. Experimental findings are discussed to illustrate biogenetic interrelationships between ergoline alkaloids in *I. violacea* in the light of earlier studies on ergot alkaloids.

THE FORMATION of alkaloids during various stages of plant growth has been investigated in several species of medicinal importance, notably in *Solanaceae* (1, 2). Our interest in the ontogeny of alkaloids in seeds of *Ipomoea violacea* stems from earlier work which dealt with the examination of various commercial samples of morning glory seeds for alkaloids, the toxicity of crude seed extracts, and the composition of the lipid fraction of these seeds (3–6). The active principles of *I. violacea* can be classified as ergoline derivatives, most of which occur also in ergot. Extensive biogenetic work elucidated the origin of the ergoline ring in ergot alkaloids (7, 8). Biogenetic interrelationships between these alkaloids have also been investigated (9–11). Gröger *et al.* (12) reported on the biogenesis of ergoline derivatives in *I. rubro-caerulea* Hook, which is considered to be synonymous with *I. violacea*, and found that in young excised plants L-tryptophan and mevalonic acid can be considered precursors of the ergolines, a pathway proposed earlier for the biogenesis of ergot alkaloids. Taber and Heacock (13) reported that in the seeds of *Rivea corymbosa*, another species of the *Convolvulaceae*, ergoline alkaloids are concentrated in the embryo. The alkaloids were also found in vegetative tissues of mature plants of *R. corymbosa* (14) and *I. violacea* (15) but in lower concentrations than in the seeds. The morphology of the seed development of *I. rubro-caerulea* and *I. violacea* has been described (16, 17), but no chemical studies during ontogeny can be found in the literature. Investigation of the seeds is of importance because frequently their misuse, stimulated by accounts of the alleged psychotomimetic action of the active principles, has been reported (18–20). A recent study by Isbell and Gorodetzky (21) with former morphine addicts indicates that the ergolines in *I. violacea* have predominantly sedative properties. It is the purpose of this paper to report on changes of the alkaloid content in the seeds of *I. violacea* which occur during ontogeny of the plant.

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EXPERIMENTAL

Procedure.—Plants employed in this study were grown under greenhouse conditions. Seeds from a commercial source of *I. violacea* L. (heavenly blue) were germinated in culture flats (March 10, 1965). After 10 days the plants were transplanted into clay pots containing sandy loam and then fed with 20-20-20 fertilizer at regular intervals. Three plants (*A*, *B*, and *C*) were selected for this study. Seed collections were made for each plant separately. Greenhouse temperatures were as follows: sunny days, 70° F.; cloudy days, 65° F.; nights, 60° F. No artificial light was employed. The first flowers appeared on June 10, 1965. From then on pollination was carried out daily by hand during the time of maximal opening of the corolla (10 a.m. to 12 p.m., depending on daylight conditions). The pollinated flowers were marked. Plants yielded from 1 to 22 flowers daily. Collection of fruit capsules was begun 10 days after fertilization and continued at 10-day intervals for 57–62 days when maturity was reached. Seeds were freed from locules of the fruiting body, and placed in a deep freeze refrigerator (−25°) under nitrogen within 1 hr. after collection. One to seven days after harvesting, the seeds were freeze-dried to constant weight and stored under nitrogen at −25° until used for analysis. There were 36 to 305 seeds per plant harvested at each stage of collection.

Alkaloid Analysis.—The dry seeds were ground in a Wiley laboratory mill to pass a 0.037-in. screen and defatted with petroleum ether (b.p. 30–60°). Analyses for total alkaloids, lysergic acid amide (LAA), isolysergic acid amide (isoLAA), and clavines, and examinations of alkaloidal patterns obtained by thin-layer chromatography (TLC) were carried out by methods described previously (3, 6). Samples (0.25–1 Gm.) of dried seeds were analyzed in duplicate for total alkaloids. Values for LAA, isoLAA, and clavines were obtained by averaging

results from densitometric scannings of 2–4 thin-layer plates per sample, each containing nine spots of test material. TLC systems used were: sytem *1* (alumina G and $CHCl_3$–EtOH, 96:4), system *2* (Silica Gel G and acetone–piperidine, 9:1) for densitometric method; system *3* (Silica Gel G and acetone–ethylpiperidine, 9:1); system *4* (alumina G and acetone–ethylpiperidine, 9:1); system *5* (Silica Gel G and acetone–ethyl acetate–dimethylformamide, 5:5:1).

RESULTS

Flower and Seed Production.—The pattern of flower and seed production was very similar in all three plants. While the first flowers appeared during the 13th week after sowing, the first peak of flower production was reached within 3 weeks after flowering had begun. At that stage approximately 100 flowers per plant were produced each week. Until the end of July when flower production had subsided the plants had produced 284, 278, and 291 flowers, respectively. During this period they produced 230, 238, and 228 fruit capsules. Seed samples derived from flowers fertilized from June 6 to July 31 are referred to as first crop. After the first a second, smaller, flowering peak developed. Seed samples derived from flowers fertilized from August 11 to September 23 are referred to as second crop. Since the second crop was only 12.5% of the first, the mature seeds in this group only were collected. Fresh and dry weights of the maturing seeds are plotted in Fig. 1. The fresh weight increased rapidly after fertilization, reached a plateau after 20 days, and fell rapidly during the latter stage of maturity, while the dry substance rose gradually until the 50th day after fertilization, leveling off during the last 10 days of the maturation process.

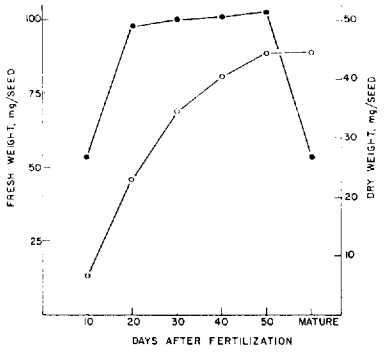

Fig. 1.—Fresh and dry weight of average seed of *I. violacea* L. (heavenly blue) during increasing maturity. Average from three plants. Key: ●, fresh weight, mg.; O, dry weight, mg.

Total Alkaloids.—The total ergoline alkaloids in seeds with increasing maturity are represented in Fig. 2. Their concentrations are highest during the early stage of seed development. From the 30th day after fertilization until full maturity was reached the alkaloid content in the dry seed substance decreased slowly. If calculated on a per seed basis (Fig. 3), the total ergoline alkaloids increased rapidly from the 10- to the 40-day samples and then dropped slowly again as maturity increased. Values for the second crop were always higher than those for the first crop.

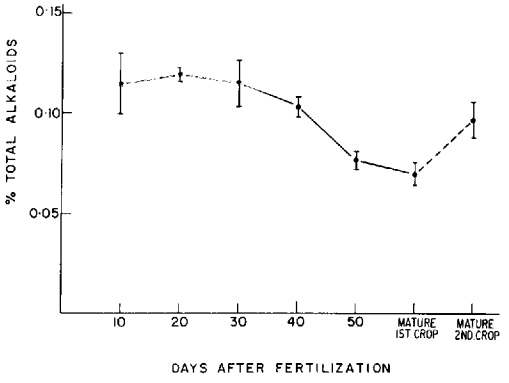

Fig. 2.—Total ergoline alkaloids as per cent of dry seeds during increasing maturity. Average value from three plants and standard deviation are indicated schematically.

Individual Alkaloids.—Results of the determination of LAA, clavines, and isoLAA in seeds collected during increasing maturity are given in Table I and Fig. 4.

As shown in Table I all three alkaloidal components were present from the first sampling date until maturity of the seed was reached. Their relative composition, however, changed. In the early stages after fertilization the clavines were most abundant. Between the 20th and 30th day after fertilization LAA became the predominant constituent which it remained until full seed maturity was reached. The steady decrease of clavine alkaloids after the 20-day sampling can been seen in Fig. 4. LAA reached its maximum concentration at about the 40th day after fertilization, and

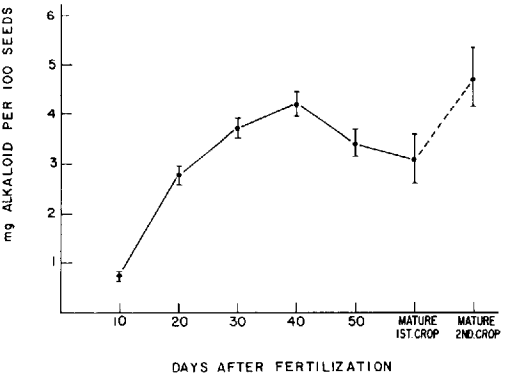

Fig. 3.—Total ergoline alkaloids (mg.) in 100 dry seeds during increasing maturity. Average value from three plants and standard deviation are indicated schematically.

TABLE I.—LYSERGIC ACID AMIDE, CLAVINE ALKALOIDS, AND ISOLYSERGIC ACID AMIDE (mg.) PER 100 DRY SEEDS OF THREE PLANTS OF *I. violacea* L. (HEAVENLY BLUE)

Days After Fertilization	Compd.	Plant *A*	Plant *B*	Plant *C*
10	LAA	0.23	0.29	0.24
	Clavines	0.35	0.37	0.31
	isoLAA	0.08	0.10	0.09
20	LAA	0.86	1.13	1.19
	Clavines	1.23	1.17	1.28
	isoLAA	0.31	0.47	0.55
30	LAA	1.77	1.52	2.31
	Clavines	1.01	1.43	1.51
	isoLAA	0.47	0.88	0.86
40	LAA	2.25	2.15	2.66
	Clavines	0.93	1.21	1.07
	isoLAA	0.72	1.03	0.87
50	LAA	1.72	2.09	2.08
	Clavines	0.87	0.96	0.67
	isoLAA	0.43	0.63	0.53
Mature 1st crop	LAA	1.86	2.08	1.44
	Clavines	0.85	0.81	0.57
	isoLAA	0.41	0.43	0.57
Mature 2nd crop	LAA	3.22	3.42	2.31
	Clavines	1.11	1.00	1.16
	isoLAA	0.50	0.57	0.65

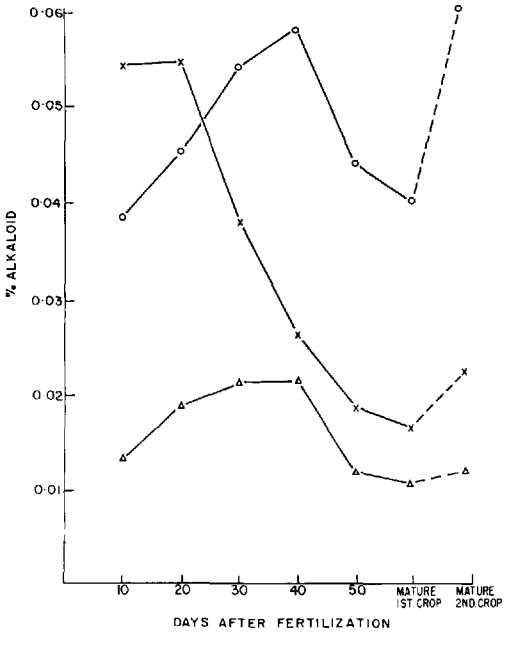

Fig. 4.—Content of lysergic acid amide (LAA), clavines and isolysergic acid amide (isoLAA) in maturing seeds expressed in per cent alkaloid in dry seeds. Averages from three plants are plotted. Key: O, LAA; ×, clavines; △, isoLAA.

its concentration decreased in the later stages of maturity. The change in concentration of isoLAA was similar to that of LAA during increasing maturity but on a lower level. IsoLAA was always the smallest component of the three groups investigated. It too reached its maximum concentration about halfway through maturity. Ergometrine included in the values for clavines, was also present during all stages of seed development. In some cases it was measured separately from the clavine alkaloids. Thus, in seed samples of plant *C* obtained 30 days after fertilization and in the mature seed of plant *B*, 33.0 and 39.4%, respectively, of the clavine fraction consisted of ergometrine. For plant *A* a complete set of ergometrine analyses was carried out. Comparison of results shown in Table II with those recorded in Fig. 2 demonstrates that the decrease of the percentage of ergometrine in the dry seeds was accompanied by a decrease of total alkaloids during seed development. On a per seed basis the ergometrine content is rather constant following the 20th day of fertilization. The ergometrine concentration in the clavine fraction is, however, higher in the more mature samples as compared to the samples closer to the fertilization stage.

The increase in alkaloids in the second crop samples is due to higher values in all three alkaloidal groups as can be seen in Table I and Fig. 4.

Qualitative TLC Patterns.—More information on the clavine alkaloids, which in the densitometric method were estimated as one group, could be

TABLE II.—ERGOMETRINE CONTENT IN SEEDS OF PLANT *A* DURING INCREASING MATURITY

Age of Seeds, Days	10	20	30	40	50	Mature
Ergometrine in dry seeds, %	0.0108	0.0132	0.0107	0.0078	0.0064	0.0061
Ergometrine in 100 dry seeds, mg.	0.06	0.29	0.36	0.31	0.28	0.27
Ergometrine in clavine fraction, %	17.4	23.8	35.7	33.0	32.2	32.0

obtained by chromatography in other TLC systems. Chromatography of the extracts of seeds in various stages of maturity indicated that the changes observed in the clavine group were mainly due to a decrease of the chanoclavine content (Fig. 5) with increasing seed age. This was also observed in systems *1* and *4*. The sum of lysergol, elymoclavine, and penniclavine, which compounds were present in trace amounts only, was lower than the content of ergometrine in all stages of seed development. Lysergic acid methyl carbinolamide, if present, would also be included with the clavine group in the TLC system used for densitometry. R_f values obtained in five systems for this carbinol are shown in Table III. The carbinol can be separated from the other known alkaloidal constituents of *I. violacea* using systems *3* or *5* after rechromatography to separate it completely from penniclavine. As indicated in Fig. 5, no appreciable amounts of the carbinol could be detected at any stage of seed maturity. In addition to the known ergoline alkaloids, at least three spots were observed having higher R_f values than any other alkaloid (R_f system *5*: 0.63, 0.75, 0.79). All these spots showed blue fluorescence under U.V. light and the characteristic blue color given by ergot alkaloids following treatment with dimethylaminobenzaldehyde. Although none of them were identical with agroclavine, there were very faint spots in the agroclavine area when using systems *1*, *3*, *4*, or *5* for the analysis of some seed extracts. In the area where penniclavine is expected, only weak greyish-bluish spots developed 30 min. after spraying.

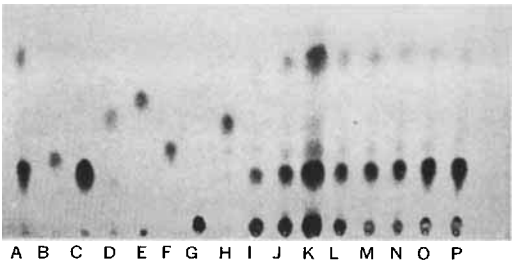

Fig. 5.—Thin-layer chromatogram of ergoline alkaloids and seed extracts of *I. violacea* in system *5*. Key: the spots from left to right are: A, LAA + isoLAA; B, lysergol; C, elymoclavine; D, penniclavine; E, agroclavine; F, ergometrine; G, chanoclavine; H, lysergic acid methylcarbinolamide (with some impurities of ergometrine and LAA). Seed extracts: I, 10; J, 20; K, 20 (3× concentration); L, 30; M, 40; and N, 50 days after fertilization; O, mature seeds (1st crop); P, mature seeds (2nd crop).

TABLE III.—R_f VALUES OF LYSERGIC ACID METHYL CARBINOLAMIDE IN FIVE TLC SYSTEMS

System	*1*	*2*	*3*	*4*	*5*
R_f value	0.30	0.36	0.55	0.23	0.38

DISCUSSION

Many investigations have been carried out on the biogenesis of ergoline alkaloids in parasitic and saprophytic cultures of various ergots or strains of *Claviceps*. The origin of the ergoline nucleus has been attributed to L-tryptophan and mevalonic acid. Several possible biogenetic interrelationships have been discussed. While Abe (22) suggested that all ergot alkaloids derive from a common precursor, a hypothetical lysergic acid aldehyde, which then would be oxidized to lysergic acid or reduced to elymoclavine, agroclavine, and chanoclavine, Rochelmeyer postulated (23, 24) that penniclavine and chanoclavine were possible precursors. The partial pathway, agroclavine → elymoclavine → penniclavine, was established by Agurell and Ramstad (10) in tracer experiments with an ergot strain isolated from *Pennisetum typhoideum*. This observation eliminated the possibility of clavine formation *via* reduction. According to Voigt (11) chanoclavine plays a key role in the development of biogenetic alkaloidal patterns as shown by experiments with rye ergot in various stages of maturation. Further contributions to the biogenetic interrelationships between various clavine alkaloids were made by Abou-Chaar *et al.* (25), Baxter *et al.* (26–28), and Agurell and Ramstad (29). The conversion of clavine alkaloids into lysergic acid alkaloids was proven in experiments with parasitic and saprophytic cultures of *Claviceps* (30, 31). The various findings in this field could partially be explained by the use of different nutrients for saprophytic cultures of fungi (32), but differences in strains producing different alkaloidal patterns have to be considered. The occurrence of alkaloids in varying concentrations during different stages of seed development in *I. violacea* could also be due to transformations of alkaloids into one another. Problems on possible transformation paths are complicated because all alkaloids occur simultaneously in all phases of seed development, and little information has yet been gained on the movement of the alkaloids in the plant. Although only limited conclusions in relation to the biogenesis of ergoline alkaloids are possible from our experimental data, findings in the much more thoroughly investigated field of ergot alkaloids could have some bearing on the elucidation of a biogenetic pathway for lysergic acid derivatives in *I. violacea*. The relatively high concentration of alkaloids and their pattern during the early stages of seed development could indicate that preformed alkaloids are predominantly deposited in the seeds, and that the seed is not necessarily the original site of alkaloid synthesis. Gröger *et al.* (12) reported in tracer experiments that while L-tryptophan and mevalonic acid were incorporated into excised *I. rubro-caerulea* plants to form ergoline alkaloids, their specific rate of incorporation into alkaloids isolated from seeds was much smaller than that into alkaloids isolated from stems. Also radioactive elymoclavine could be converted into penniclavine by detached stems of the same plant (33). Comprehensive tracer studies proving to what degree, if at all, seeds in *I. violacea* can synthesize ergoline alkaloids remain to be carried out and are not only important for finding the site of alkaloid synthesis but also for the elucidation of biogenetic interrelationships between alkaloids in this plant. Indications that chanoclavine may be the precursor of LAA and its epimer are seen in our results on individual alkaloids (Figs. 4 and 5). Ergo-

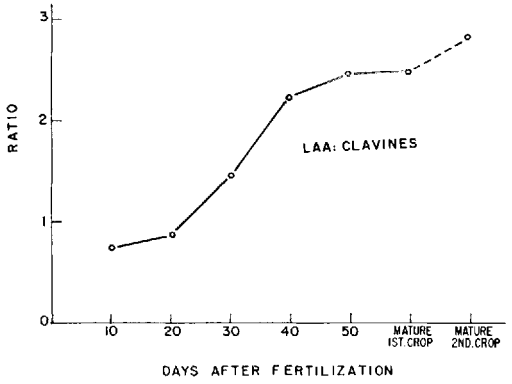

Fig. 6.—Ratio of lysergic acid amide/clavine alkaloids during increasing maturity of seeds.

metrine, on the other hand, seems to appear preferably in the middle and later period of seed development. The change of the LAA/clavine ratio (Fig. 6) would indicate a transformation of clavines (chanoclavine) into LAA during the course of increasing seed maturity. Thus, a biogenetic pathway for ergoline alkaloids in *I. violacea* analogous to that of the ergot alkaloids may be postulated: chanoclavine → (agroclavine) → (elymoclavine) → (penniclavine) → LAA; isoLAA → ergometrine → (lysergic acid methyl carbinolamide). The alkaloids shown in parentheses were found in the seeds in traces only or, in some cases, not even detected. They could, possibly, occur in greater abundance at the original site of alkaloid synthesis in the plant. The presence of mere trace amounts of lysergic acid methyl carbinolamide, which (alkaloid) was reported as one of the major components in *I. rubro - caerulea* (15) could indicate a genetic difference between this plant and *I. violacea* even though both plants are considered to be synonymous botanically. This interpretation, however, has to be used with caution since blank experiments with small amounts of carbinol showed that, under our experimental conditions, some of the applied carbinol is being decomposed.

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