Paper Chromatography of Alkaloidal Extracts of *Lobelia* Species

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Total alkaloidal extracts from four closely related *Lobelia* species, *L. cardinalis* L., *L. syphilitica* L., *L. puberula* Michaux, and *L. elongata* Small, and ten crosses and hybrids involving *L. cardinalis* and *L. syphilitica,* were examined using two-phase descending paper chromatography with formamide-ammonium formate-formic acid as the stationary phase and chloroform-benzene as the mobile phase. The chromatogram of *L. elongata* showed the presence of lobinaline, suggesting its closer relationship with *L. cardinalis.* The chromatograms of *L. cardinalis, L. syphilitica,* and *L. puberula* were different from each other, suggesting the elaboration of different alkaloids by each of these related species. Lobinaline was present in the first species and absent in the latter two species. The *L. cardinalis* × *L. syphilitica* hybrids gave chromatograms suggesting the elaboration of alkaloids characteristic of both the parent plants.

As a result of extensive chemical investigations on various plant species, especially those of economic importance, as, for example, vegetable drugs, the occurrence of "chemical races," "physiological races," or "chemical varieties" has been suggested (1, 2). These plants have similar phenotype but different genotype, and as such are identical in external characters but differ in their chemical composition. Study of chemical composition in relationship to the taxonomy of plants and the development of "chemical systematics" or "biochemical systematics" has interested many workers in recent years (3–9). Systematic chemical studies of genera and families have led to the discovery of new and sometimes unexpected structural types. The observation that certain compounds sometimes do not occur in groups of plants where their presence for botanical reasons could be expected, or that these compounds are replaced, has stimulated study of the deeper causes of such anomalies and led to the deeper understanding of the mechanisms of evolution and the later stages of biosynthetic processes.

The genus *Lobelia* has been of interest from the above point of view. *L. inflata* L. contains lobeline as the major alkaloid while *L. nicotianaefolia* Heyne contains similar alkaloids, lobelanidine being the major alkaloid (10). Bowden (11) has correlated taxonomic, ecological, and cytogenic data on 21 species of *Lobelia* L. section *Lobelia* and has observed the relationship between these species. The alkaloidal extracts from four species of *Lobelia: L. cardinalis* L., *L. syphilitica* L., *L. elongata* Small, and *L. puberula* Michaux, and ten crosses and hybrids involving *cardinalis* and *syphilitica*, have been examined by the technique of descending paper chromatography in this work.

EXPERIMENTAL

Plant Materials.—Total alkaloidal extracts of the following four species of *Lobelia* and ten hybrids were obtained.[1] The method of preparation of the extracts was as outlined by Manske (14). The quantity of dried plant material extracted and the total amount of extractive are indicated in brackets following each species or cross (Gm.). X-515: diploid, *cv.* "Illumination" × *L. cardinalis* subsp. *graminea* var. *pseudosplendens* (N. Mex.) (933-3.90); X-517: *cv.* "Queen Victoria" × *L. cardinalis* subsp. *cardinalis* (584-2.55); X-518: *cv.* "Queen Victoria" × *L. cardinalis* subsp. *cardinalis* (677-2.50); X-537: tetraploid, from a double hybrid cross (two F_1's) involving two accessions of *L. cardinalis* subsp. *cardinalis* and two accessions of *L. syphilitica* var. *syphilitica* (545-1.52); X-520: *L. cardinalis* subsp. *cardinalis* × *L. cardinalis* subsp. *graminea* var. *pseudosplendens* (N. Mex.); X-552: *L. cardinalis* subsp. *graminea* var. *graminea* (Mexico) × *L. cardinalis* subsp. *graminea* var. *graminea* (Mexico); X-628: *L. cardinalis* subsp. *graminea* var. *graminea* (Honduras) × *L. cardinalis* subsp. *graminea* var. *graminea* (Mexico) (X-520, X-552, and X-628 were mixed) (256 + 281 + 125-3.24); X-917-S: selfed, complex tetraploid hybrid involving *cardinalis* and *syphilitica* tetraploid: X-918: same as X-917-S, but two seedlings of the parent hybrid were intercrossed to give X-918, tetraploid X-917-S and X-918 were mixed (343-1.28); X-897: tetraploid, complex parentage involving *L. cardinalis* and *L. syphilitica*, had some of the same parentage as X-537 (523-1.80); X-749: tetraploid, involving *L. cardinalis* and *L. syphilitica;* X-909: tetraploid, involving *L. cardinalis* and *L. syphilitica* (X-749 and X-909 were mixed) (179 + 390-1.35); L.C.C.

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[1] The materials were supplied by Dr. R. H. F. Manske, Dominion Rubber Co., Guelph, Ontario, and Dr. W. M. Bowden, Botany and Plant Pathology Laboratory, Department of Agriculture, Ottawa, Ontario.

403-407 (681-2.83); *L. cardinalis* var. *cardinalis* 5-6-B-S7: *L. elongata* Small, grown from seeds collected at Edenton, N. C. (591-2.42); 17-21-S1-3: diploid, mostly *cardinalis* genes but may be a hybrid, from Erfurt, Germany (681-1.08); *L. puberula* Michx.: composite sample of seedlings grown from seeds collected in North and South Carolina and Florida (196-0.35); *L. cardinalis* var. *cardinalis*; *L. syphilitica* var. *syphilitica*.

Chromatographic Procedure.—Preliminary separations showed that Whatman No. 3 chromatographic paper gave better separations, more constant R_f values, and little or no streaking.

The following two-phase system developed by Steinegger and Ochsner (12) was selected following preliminary screening. Stationary phase: 9 ml. of formamide (laboratory reagent) was dissolved in 20 ml. of acetone (distilled at 56–57° and dried over anhydrous sodium sulfate). The solution was saturated with ammonium formate (0.9 Gm. laboratory grade) and the supernatant filtered through dry filter paper. One milliliter of concentrated formic acid (98–99%) was added to the filtrate. Mobile phase: 9 parts of chloroform (analytical) was mixed with 1 part of benzene (analytical).

Whatman No. 3 paper strips, 19 cm. wide and 57 cm. long, were impregnated with the stationary phase by passing the strips through the solution in a flat dish from about 2.5 cm. above the starting line and down to the lower edge. Excess solution was blotted between filter paper sheets and the paper hung in air to remove all acetone. Six spots were applied to the paper in methanol (synthetic) solution. Approximately 400 mcg. of the total alkaloidal extract and 100 mcg. of the pure alkaloid were applied. Each strip carried one spot of lobinaline which served as a control spot. The spotted paper was then allowed to equilibrate with the vapor of the mobile phase in Pyrex glass chromatographic jars for 12 to 15 hours. The chromatogram was developed after this equilibration period until the mobile phase had run approximately 45 cm. from the starting line. The paper strips were then taken out of the jars and dried in air for one-half hour. Formamide was removed from the strips by keeping them in a hot-air oven at 90° for 2 hours. The color of the alkaloidal spots was developed by spraying with modified Dragendorff's reagent (13). The orange-red alkaloidal spots were marked off on the paper with a pencil and the R_f values of

Table I.—R_f Values of the Dragendorff-Positive Spots from the Extracts of Four *Lobelia* Species

L. cardinalis	*L. puberula*	*L. elongata*	*L. syphilitica*
0.00	0.00	0.00	0.00
0 48[a]	0.06	0.03	0.025
0.80	0.1	0.041	0.15[b]
	0.2	0.06	0.25
	0.3	0.46[a]	0.30[b]
	0.52	0.80	0.40
			0.55
			0.75

[a] Rf values for lobinaline. [b] Rf values for lophilacrine and lopheline (12, 13).

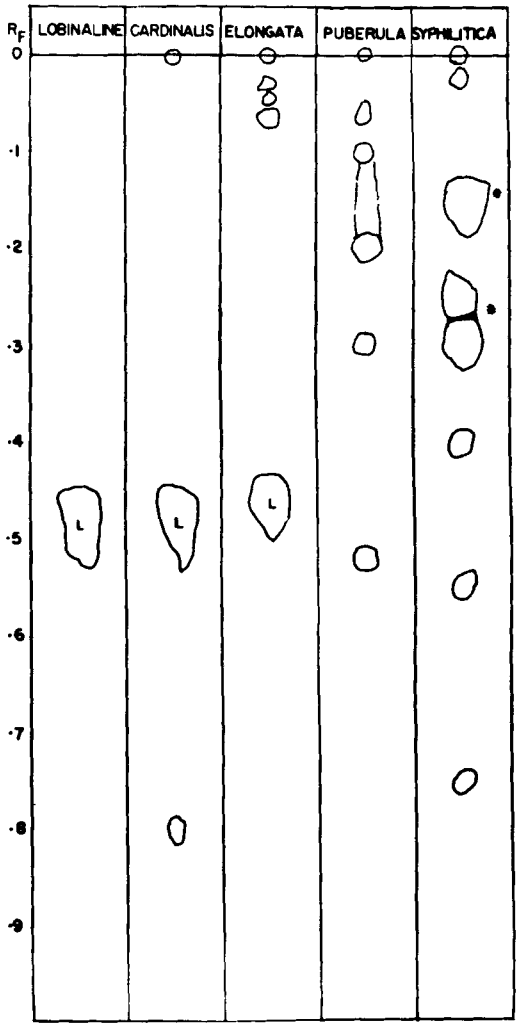

Fig. 1.—Chromatograms of total alkaloidal extracts of four *Lobelia* species. L, lobinaline; *, lopheline and lophilacrine.

Table II.—R_f Values of the Dragendorff-Positive Spots from the Extracts of the *Lobelia* Hybrids

X-515	X-517	X-518	X-537	X-520 X-552 X-628	X-897	X-749 X-909	L.C.C. 403–407	X-917 S X-918	12-21-S1-3
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.06	0.07	0.04	0.06	0.02	0.025	0.025	0.027	0.06	0.06
0.20	0.16	0.07	0.19[b]	0.06	0.08	0.07	0.07	0.15[b]	0.15[b]
0.40	0.3	0.2	0.3[b]	0.18	0.16[b]	0.18[b]	0.5[a]	0.28[b]	0.3[b]
0.52[a]	0.52[a]	0.48[a]	0.5[a]	0.50[a]	0.24[b]	0.3[b]	0.82	0.53[a]	0.54[a]
0.87	0.90	0.87[a]	0.9	0.8	0.49[a]	0.54[a]		0.89	0.72
					0.75	0.87			

[a] Rf values for lobinaline. [b] Rf values for lophilacrine and lopheline (12, 13).

each noted. (See Tables I and II; Figs. 1, 2, and 3.) The R_f values given are only a guide and are average values of about 10 determinations. The variables governing R_f values are numerous and, for this reason, the locator spot of a solution of pure crystalline lobinaline was applied on each strip.

RESULTS

The chromatograms and R_f values (Figs. 1, 2, and 3 and Tables I and II) were compared with each other on the basis of the presence or absence of lobinaline (14). Other alkaloids in these species have not been isolated or characterized to any extent. *L. cardinalis* L. gave three spots, and that corresponding to lobinaline at R_f 0.46 to 0.53 was the most prominent (Fig. 1). *L. syphilitica* L. gave eight spots, and the two prominent spots at R_f 0.16 and 0.3 have been attributed to the presence of two liquid alkaloids, lopheline and lophilacrine, respectively (15). This species did not appear to contain lobinaline. *L. puberula* Michaux gave six small spots and the chromatograms would indicate an absence of lobinaline in this species also. The chromatograms appeared to be different from those of the extracts from the other three species (Fig. 1). *L. elongata* gave six spots. The prominent spot at R_f 0.46 was eluted out and rechromatographed after mixing with pure lobinaline. Only one spot was obtained, thus indicating the presence of lobinaline in this species.

Chromatograms of the extracts from crosses involving *L. cardinalis* varieties alone, X-515, X-517, X-518, X-520 + X-552 + X-628, and L.C.C. 405-407 (Fig. 2) showed the presence of lobinaline. In these cases there were a greater number of alkaloids (5 to 6 spots) than the three elaborated by *L. cardinalis* L. The hydrids involving *L. cardinalis* L. and *L. syphilitica*, X-537, X-917-S + X-918, X-897, X-909 + X-749 (Fig. 3), gave chromatograms showing spots due to lobinaline and also due to lopheline and lophilacrine. The extract 12-21-S1-3 gave spots corresponding to lobinaline, lophilacrine, and lopheline (Fig. 3).

DISCUSSION

According to Bowden (11), *L. elongata* Small appears to have originated by the crossing of a *puberula*-like ancestor and some other closely related species. The presence of lobinaline and the chromatograms in general with this species would appear to indicate a closer relationship to *cardinalis* than to *puberula*. Bowden has also observed that although strong morphological differences distinguish *L. cardinalis* from *L. syphilitica*, cytogenetically these two species are certainly related, *L. cardinalis* having been derived by some mutational process from a *syphilitica*-like ancestral species, the

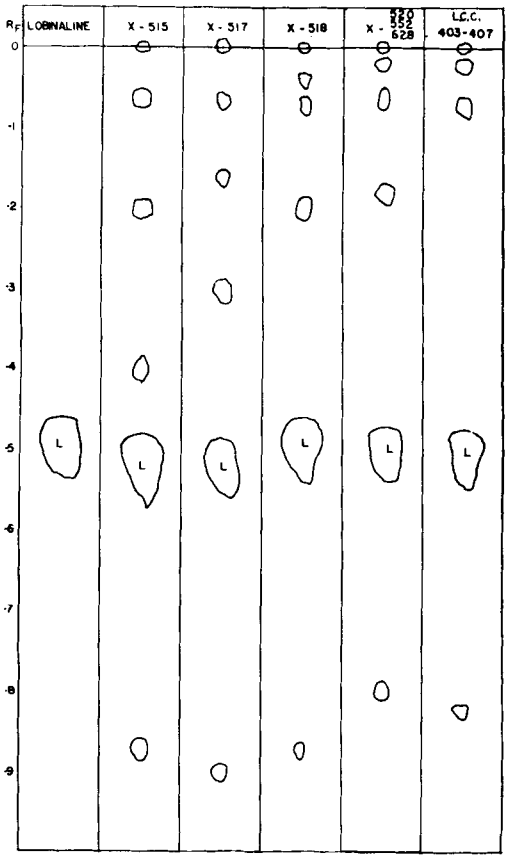

Fig. 2.—Chromatograms of total alkaloidal extracts of crosses involving *L. cardinalis* L. L, lobinaline.

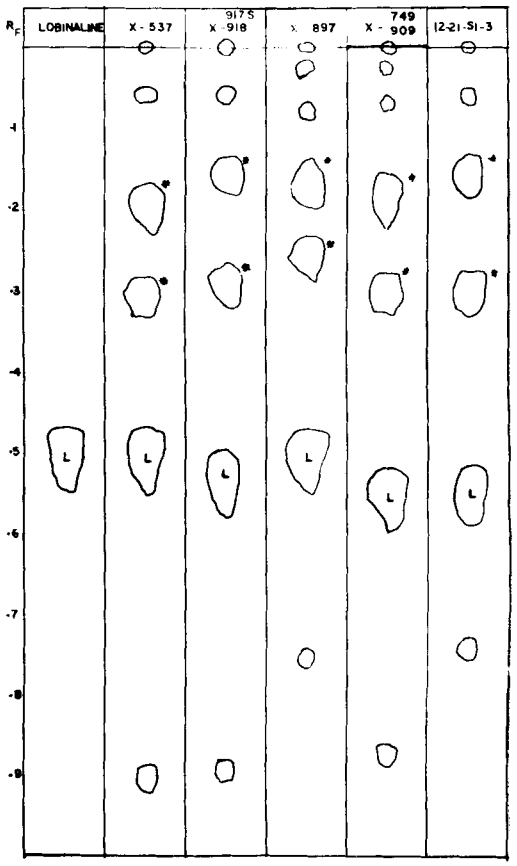

Fig. 3.—Chromatograms of total alkaloidal extracts of *L. cardinalis* and *L. syphilitica* crosses. L, lobinaline; *, lopheline and lophilacrine.

mutational changes involving changes in chromosome structure. The chromatograms of these two species showing the occurrence of lobinaline in the first species and its absence in the latter would suggest that these mutational changes have also resulted in changes in alkaloidal composition and in structural type in these two species. *L. syphilitica* and *L. puberula* have been shown to be cytogenetically related by Bowden. The chromatograms of these two species do not seem to show any close relationship. The *cardinalis* × *syphilitica* hybrids produce alkaloids from both the parent plants. These observations on *L. syphilitica* and the *cardinalis* × *syphilitica* hybrids support and extend the observations of Steinegger, *et al.* The plant 12-21-S1-3 (Fig. 3) was observed to be a diploid plant with mostly *cardinalis* genes but which might have been a hybrid. The chromatograms having spots due to lobinaline and lopheline and lophilacrine indicate that the sample was a hybrid involving *cardinalis* and *syphilitica*.

The chromatograms of *L. cardinalis* L. (Fig. 1 and Table I) show three spots: R_f 0.00, 0.48, and 0.80. Kaczmarek and Steinegger (16) obtained only two spots, R_f 0.45 and 0.86, with fresh extracts of the species. However these same authors observed an increased number of spots (5–8) with a 6-year-old petroleum ether extract.

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Bisulfite Reduction of *p*-Nitrobenzyl Alcohol

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Bisulfite reduction of *p*-nitrobenzyl alcohol appears to follow a Piria mechanism leading to formation of α-hydroxy-6-sulfoamino-*m*-toluenesulfonic acid. Stoichiometry of the reaction involves one mole of nitro compound and three moles of bisulfite; pH profile of the initial rate of the stoichiometric reaction has been studied. Preliminary kinetic studies indicate an apparent heat of reaction of about 22 Kcal. mole^{-1}. This complicated sequential reduction reaction appears to involve two mechanistic pathways: a rate-determining initial S_N1 reaction dominates at pH 4, while at pH 7 the initial rate-determining reaction appears to possess typical S_N2 characteristics.

BISULFITE REACTIVITY toward the *p*-nitrobenzyl alcohol moiety of chloramphenicol was demonstrated during an investigation of the stability of the antibiotic in the presence of the thio compound (1). Complexity of the antibiotic molecule made evaluation of the kinetic results rather difficult and, for this reason, *p*-nitrobenzyl alcohol was employed as a model compound for the antibiotic so that certain mechanistic details of the reaction could be evaluated. Spectrophotometric and chromatographic evidence obtained during the kinetic study of both the antibiotic and the model compound indicated involvement of nitro reduction with formation of a very polar product; this served to differentiate the reaction from that which occurs between nitrobenzyl halides and sulfite (2). Nitro reduction plays only a minor role under reaction conditions which lead to near-quantitative yields of the nitrobenzyl sulfonic acid from the corresponding nitrobenzyl halides.

The reaction of either *p*-nitrotoluene or *p*-nitrobenzoic acid with sulfite in dilute aqueous solution under conditions similar to those employed with the *p*-nitrobenzyl alcohol leads to formation of both sulfoamino and sulfoaminosulfonic acid compounds (3, 4). This reduction-sulfonation reaction is known as the Piria reaction (3). The mechanism of the Piria reaction for *p*-nitrotoluene analogs of *p*-nitrobenzyl alcohol and other aromatic nitro compounds has been treated

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