Chromatographic Study of the Alkaloids of *Aquilegia formosa*

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Chromatographic investigation of alkaloid fraction from the roots of *Aquilegia formosa* Fisch. (*Ranunculaceae*) revealed the presence of five alkaloidal spots. Two of them were identified as magnoflorine and berberine. A thin-layer chromatographic procedure was developed which effectively separated berberine, palmatine, and jatrorrhizine.

A PHYTOCHEMICAL investigation of plants considered to be of medicinal value by certain Indian tribes of the Northwest, particularly those of the Warm Springs Reservation, Ore., revealed the presence of alkaloids in the roots of *Aquilegia formosa* Fisch.

An ethnobotanical survey conducted by French (1) noted that numerous species of *Aquilegia* have been widely used by the Indians of North America for a host of medicinal purposes. The reputed medicinal uses include diuretic and analgesic activity, treatment of diarrhea or smallpox, a stimulating tea, etc. Species of *Aquilegia* were employed not only as medicines but also as perfumes (2), and were used in certain magical rites. Hitchcock *et al.* (3) indicate that there are approximately 70 species of *Aquilegia* native to the north temperate zones. They are commonly referred to as "columbines," and many are available as horticultural varieties.

A comparative paper chromatographic screen of 10 horticultural varieties of *Aquilegia* was conducted by Winek *et al.* (4). Their screen did not include *A. formosa*. From the entire group, three alkaloids were established—magnoflorine, berberine, and aquileginine. Chromatography also revealed other alkaloids, but their identities were not established. The presence of palmatine remained equivocal because the solvent systems used did not separate this alkaloid from berberine. An investigation of *A. hybrida* by this same group (5) revealed eight alkaloids in this species. Three of the isolated alkaloids were identified as berberine, magnoflorine, and aquileginine. The others remain unknown.

Since preliminary studies indicated alkaloids in *A. formosa*, and readily available quantities could be collected in the wild, the present study was undertaken. The objectives of this investigation were to identify the alkaloids in this species and to develop a thin-layer chromatographic procedure to identify the alkaloids encountered.

EXPERIMENTAL

Collection and Extraction.—*A. formosa* was collected at the flowering stage during the month of May in Benton County, Ore. The roots were separated, cleaned, and dried in a forced-air dryer at 48° for 5 days. The dried roots were ground to a coarse powder in an Abbé mill. A 2.36-Kg. quantity of this material was continuously extracted with alcohol in a Soxhlet extractor until the extractive gave a negative test with Mayer's reagent. The extract was concentrated to a syrupy residue in a flash evaporator at 40° under reduced pressure. The residue (approximately 600 Gm.) was stored in a refrigerator while portions of it were investigated chromatographically.

Separation of Alkaloidal Fraction.—A 200-Gm. portion of the extract was dissolved in a solution containing 100 ml. of 2% hydrochloric acid and 500 ml. of distilled water. A 2% solution of ammonium reineckate was then added until precipitation ceased (approximately 400 ml.). The mixture was refrigerated for 6 hr., the liquid decanted, and the residue dried *in vacuo*. The dried residue was then taken up in warm acetone (250 ml.), and an equal volume of distilled water was added. To this mixture was added 430 ml. of 0.6% silver sulfate to precipitate silver reineckate (6). The mixture was filtered and the filtrate saved. The precipitate was washed with several portions of 50% acetone (total 500 ml.) and the washings added to the original filtrate. The filtrate was then concentrated to a thick syrup in a flash evaporator.

Chromatographic Procedures.—The initial separation and identification procedures utilized were modified techniques of Winek *et al.* (5). The concentrate described previously was adsorbed onto 28.0 Gm. of activated alumina[1] and pulverized to a fine powder with a mortar and pestle. Ten grams of this mixture was placed atop an activated alumina column (35 × 150 mm.). The column was then developed successively with benzene, chloroform, several varying chloroform–methanol mixtures, and finally methanol alone (Table I, AB). Each fraction was concentrated to approximately 10 ml. in a flash evaporator and subjected to analysis using paper chromatography. The extracts were spotted on Whatman No. 2 filter paper and developed with the upper phase of *n*-butanol–acetic acid–water (BAW 5:1:4) using the ascending technique, or *n*-propanol–ammonium hydroxide–water (PAW 2:1:1) employing the descending technique. The spots were revealed by examining the sheets under ultraviolet light and by spraying them with Dragendorff's reagent (7). The results obtained in this manner were not completely satisfactory, and subsequent analyses were conducted with thin-layer chromatography (TLC). TLC plates of Silica Gel G[2] were prepared according to standard techniques. Several recommended solvent systems for alkaloids (8) were tried. Effective separation of alkaloidal constituents was not realized. The combination of systems finally utilized were BAW (4:1:5) upper phase, BAW (4:1:1), and PAW (2:1:1). The spots were located as previously de-

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[1] Alumina activated, chromatographic grade, Matheson, Coleman and Bell, East Rutherford, N. J.

[2] According to Stahl, Brinkmann Instruments, Inc., Westbury, Long Island, N. Y.

TABLE IA.—PAPER CHROMATOGRAPHY OF COLUMN FRACTIONS AND REFERENCE COMPOUNDS[a]

Fraction	Solvent	Vol., ml.	Paper Chromatography: BAW (5:1:4) U.V.	BAW (5:1:4) *Rf*	PAW (2:1:1) U.V.	PAW (2:1:1) *Rf*
1	Benzene	500	..	...	..	...
2	Chloroform	500	..	...	..	...
3	$CHCl_3$–MeOH 97:3	500	..	...	..	...
4	$CHCl_3$–MeOH 94:6	1500		Traces		Traces
5	$CHCl_3$–MeOH 88:12	600	B	0.46	B	0.52
			B	0.56	B	0.59
			Y	0.75	..	0.87
			..	0.89	..	...
6	$CHCl_3$–MeOH 82:18	500	B	0.46	B	0.49
			B	0.57	B	0.57
			Y	0.73	..	0.87
			..	0.88	..	...
7	$CHCl_3$–MeOH 75:25	500	B	0.52	B	0.52
			B	0.62	B	0.61
			Y	0.71	..	0.87
			..	0.89	..	...
8	Methanol	500	..	Traces ...	..	Traces ...
	Reference Compd.					
	Magnoflorine		B	0.56	B	0.47
	Jatrorrhizine		Y	0.68	Y	0.56
	Palmatine		Y	0.68	Y	0.68
	Berberine		Y	0.68	Y	0.60

[a] BAW (5:1:4), *n*-butanol–acetic acid–water, 5:1:4 (upper phase); BAW (4:1:5), *n*-butanol–acetic acid–water, 4:1:5 (upper phase); BAW (4:1:1), *n*-butanol–acetic acid–water, 4:1:1; PAW (2:1:1), *n*-propanol–ammonium hydroxide–water, 2:1:1. Detection, U.V. fluorescence and Dragendorff's reagent: B, blue; Y, yellow.

TABLE IB.—THIN-LAYER CHROMATOGRAPHY OF COLUMN FRACTIONS AND REFERENCE COMPOUNDS[a]

Fraction	Solvent	Vol., ml.	Thin-Layer Chromatography: BAW (4:1:5) U.V.	BAW (4:1:5) *Rf*	PAW (2:1:1) U.V.	PAW (2:1:1) *Rf*	BAW (4:1:1) U.V.	BAW (4:1:1) *Rf*
1	Benzene	500	..	...	..	...	..	...
2	Chloroform	500	..	...	..	...	..	...
3	$CHCl_3$–MeOH 97:3	500	..	...	..	...	..	...
4	$CHCl_3$–MeOH 94:6	1500		Traces		Traces		Traces
5	$CHCl_3$–MeOH 88:12	600	Y	0.02	Y	0.60	Y	0.01
			B	0.07	B	0.63	..	0.08
			..	0.19	..	0.66	B	0.12
			B	0.24	..	...	..	...
			Y	0.35	Y	0.70	Y	0.32
6	$CHCl_3$–MeOH 82:18	500	Y	0.02	Y	0.60	Y	0.02
			B	0.05	B	0.64	..	0.07
			..	0.15	..	0.66	B	0.12
			B	0.21	Y	0.69	Y	0.32
			Y	0.31	..	...	..	...
7	$CHCl_3$–MeOH 75:25	500	Y	0.02	Y	0.62	Y	0.02
			B	0.07	B	0.64	..	0.08
			..	0.19	..	0.67	B	0.13
			B	0.24	..	...	..	...
			Y	0.35	Y	0.70	Y	0.33
8	Methanol	500		Traces		Traces		Traces
	Reference Compd.							
	Magnoflorine		B	0.24	B	0.64	B	0.12
	Jatrorrhizine		Y	0.42	Y	0.66	Y	0.33
	Palmatine		Y	0.34	Y	0.68	Y	0.26
	Berberine		Y	0.36	Y	0.68	Y	0.32

[a] See *Footnote a*, Table IA.

scribed. The results are summarized in Table I, AB. The U.V. fluorescence was noted only for those compounds which gave orange spots with Dragendorff's reagent.

Presence of Magnoflorine and Berberine.—Winek *et al.* (4, 5) reported the presence of magnoflorine and berberine in several *Aquilegia* species. Based on preliminary chromatography, it appeared that these alkaloids were also present in *A. formosa*. A portion of column fraction 5 (Table I, AB) was spotted heavily in three different channels on Silica Gel G TLC plates. Authentic magnoflorine[3] was spotted

[3] The authors are grateful to Professor J. L. Beal, School of Pharmacy, Ohio State University, Columbus, for authentic samples of magnoflorine, berberine, palmatine, and jatrorrhizine.

TABLE II.—RESULTS OF CO-SPOTTING EXPERIMENTS

Solvent	R_f Values					
	Eluate	Eluate + Magnoflorine Chloride	Magnoflorine Chloride	Eluate	Eluate + Berberine Chloride	Berberine Chloride
BAW (4:1:5)	0.22	0.22	0.22	0.36	0.35	0.36
PAW (2:1:1)	0.64	0.63	0.64	0.70	0.72	0.72

in a fourth channel. The plates were developed in BAW (4:1:5). The three zones possessing blue fluorescence and corresponding to magnoflorine (R_f 0.23) were removed from the plate by means of a zone extractor and eluted with 10 ml. of hot methanol. The solution was reduced to a small volume (approximately 0.1 ml.) and divided into two portions. A chromatoplate was prepared containing the one portion of eluate and magnoflorine spotted individually and one spot containing the other portion of the eluate plus magnoflorine. The plates were developed in PAW (2:1:1). Examination of the plate under U.V. light or spraying with Dragendorff's reagent (blue fluorescence under U.V., orange spot with Dragendorff's reagent) revealed that the eluate and the co-spot of eluate plus magnoflorine gave only one zone which had an identical R_f value to that of reference magnoflorine. Similarly, fraction 5 was chromatographed in PAW (2:1:1). The blue fluorescent zones corresponding to known magnoflorine (R_f 0.65) were extracted and eluted with hot methanol and the co-spotting experiment was run using BAW (4:1:5). Comparable results were obtained.

The presence of berberine was established in an identical manner. In this instance, column fraction 7 was used since visual inspection of preliminary chromatograms indicated higher concentration of the suspected berberine in this fraction. Yellow fluorescent zones corresponding to reference berberine [R_f 0.39 and 0.75 for chromatoplates developed in BAW (4:1:5) and PAW (2:1:1), respectively] were extracted and eluted with methanol. Concentrated eluates were co-chromatographed with berberine in a manner similar to that for magnoflorine. The results of these experiments are summarized in Table II.

TLC plates of fractions 5 and 7 were prepared and developed in BAW (4:1:1). Solutions of reference magnoflorine and berberine chlorides were spotted singly and in combination with extracts 5 and 7, and these were compared to plates of extracts without additions. Detection of spots was carried out as before. The procedure revealed the presence of both alkaloids.

Other Alkaloids.—Preliminary paper chromatography revealed the presence of other alkaloids in fractions 5 and 7. When these fractions were chromatographed in the TLC system, additional spots were noted (Table I). These spots were not identified, but co-chromatography experiments using the three solvent systems described earlier indicated that they were not palmatine or jatrorrhizine.

DISCUSSION

Winek *et al.* (4, 5) reported the presence of alkaloids in 10 species of *Aquilegia* which, however, did not include *A. formosa*. On the basis of chromatography, particularly TLC, the presence of at least five well-defined alkaloids has been demonstrated. Consistent with the results of Winek *et al.* (4, 5), *A. formosa* also contained magnoflorine as the principal alkaloid and berberine as one of the minor alkaloids. Since the majority of the conventional solvent systems (8) failed to provide effective separation of these alkaloids (perhaps because of their quaternary nature), two systems—namely, BAW (4:1:5, acidic) and PAW (2:1:1, alkaline)—were used during co-spotting experiments. In all instances, the alkaloidal mixture along with an authentic sample of either magnoflorine or berberine was run in one system and the subsequent co-chromatography was run in the other. Thus, the eluted spot and the authentic sample were subjected to an entirely new environment. In every case there was no separation of the co-spot.

Berberine and palmatine could not be distinguished by TLC employing BAW (4:1:5). However, BAW (4:1:1) separated these two closely related alkaloids cleanly. Co-chromatography using the combination of three TLC systems (Table I, AB) eliminated the presence of palmatine in *A. formosa*. In a similar manner, the presence of jatrorrhizine in the alkaloid mixture was also eliminated. It will be necessary to isolate adequate quantities of the other alkaloidal substances in order to establish their identity.

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